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# MONOGRAPHS ON PHYSIOLOGY

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AND

A. V. HILL, Sc.D., F.R.S.

## GENERAL PREFACE.

THE noblest memorial to ERNEST STARLING is in the hearts of those whom he served by his fearless generosity and his splendid friendliness. His many and varied writings, his constructive and organising power, the pupils whom he sent forth, and the fellowship which radiated from him to distant lands, will not soon allow his name to be forgotten. Here, however, it is fitting to record that for eleven years he edited these "Monographs on Physiology." "In no science," he wrote, "is the advance at any one time general. Some sections of the line are pushed forward, while other parts may remain for years with little movement, until in their turn they are enabled to progress in consequence of the support afforded by the advance of adjacent sections." "In the present series," he continued, "it is intended to set out the progress of physiology in these chapters in which the forward movement is the most pronounced." Starling's strategic insight often served his chosen science well. In attempting to continue these monographs we shall hope to be able in some measure to perpetuate this aspect of his work.

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# THE INTERNAL SECRETIONS OF THE OVARY

BY

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## AUTHOR'S PREFACE

THIS book has been written with the object of bringing together the more important facts bearing upon the internal secretions of the ovary. This subject now forms a vigorous growing-point of physiology, and its innate complexity, combined with the present activity of research workers, makes the presentation of a coherent picture difficult. Nevertheless, while many aspects must as yet be dealt with tentatively, my aim has been to bring forward in their natural relevance and in due proportion, the observational and experimental data which comprise our knowledge of the ovary as an endocrine organ.

The more general sections of the book, notably the chapter on the morphology of the female reproductive organs, are merely introductory. No useful purpose could be served by reproducing in detail what is found in numerous anatomical and histological text-books. The morphological aspect of the œstrous cycle is of necessity dealt with fairly fully, but only those species are considered which have been studied in some detail. The remaining chapters on the endocrine control of the female reproductive organs are intended, however, to be tolerably complete, both as regards fact and hypothesis. The bibliography has been designed to include as much as possible of the literature up to the end of 1928.

My obligation to workers in this and similar fields is unbounded. The development of my own research has largely been directed by the critical interest of Dr. F. H. A. Marshall, F.R.S., whose writings form the groundwork of the subject, and who has most kindly read the proofs and allowed me to use certain illustrations from his *Physiology of Reproduction*. I am deeply conscious, also, of my debt to Prof. C. Lovatt Evans, F.R.S., and Prof. J. P. Hill, F.R.S., for their continued advice and encouragement. The work on X-ray sterilization has been

made possible by the kindness of Prof. G. Elliot Smith, F.R.S., in allowing me facilities in his Department, and by the interest of Dr. H. A. Harris.

Mr. J. Hammond, in addition to allowing me to reproduce Figs. 10 and 17, has also given me the benefit of his wide experience. To Dr. J. H. Burn I am indebted for permission to reproduce Figs. 68 and 69, while Mr. W. Shaw has kindly supplied me with the human material shown in Figs. 4, 5 and 35.

Table 3 and Figs. 14, 18 and 63 respectively have been included by the courtesy of Prof. H. M. Evans, Prof. Carl Hartman, Prof. G. W. Corner, and Prof. B. Zondek, while I am particularly grateful to Dr. P. E. Smith and Dr. E. T. Engle for the illustrations in Figs. 59, 60 and 61. To all of these I would offer my best thanks.

Finally, I would take this opportunity of thanking most sincerely my friends and collaborators, Dr. F. W. R. Brambell, Mr. G. F. Marrian, Mr. C. W. Bellerby, Miss U. Fielding, Dr. A. R. Fee, and Mr. S. Zuckermann, who have taken a large part in the research upon which this monograph is based, and who have assisted me with the preparation of the book.

I have been fortunate in obtaining permission from the Council of the Royal Society, from the Cambridge University Press, and from the Editors of the *Quarterly Journal of Experimental Physiology* and of the *Lancet*, to reproduce various illustrations and figures.

The new illustrations are the work of Mr. F. J. Pittock.

A. S. PARKES.

UNIVERSITY COLLEGE,  
LONDON,  
1929.

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## CHAPTER I

### THE DIFFERENTIATION OF THE SEXES

THE sexes are distinguished primarily by the presence of ovary or testis, *i.e.* by the power to produce ova or spermatozoa. This production of germ cells is the essential function of the gonads, and in lower animals, where the reproductive processes are of the simplest, their only function. During the course of evolution, however, the tendency has been to limit the number of ova produced, and to provide each embryo with a greater degree of parental care. This tendency has culminated in the prolonged period of internal gestation found in mammals, and has resulted in the appearance of many organs and characters designed to facilitate reproduction. Coincidentally, a second function of the gonad has appeared, the control of the development of these accessory structures. These organs have reached a higher degree of elaboration in the female, upon whom the care of the young largely falls in most species.

In addition to the accessory organs, there are found secondary sexual characters, which, though of no direct use in the reproductive processes, are nevertheless valuable or necessary in diceious propagation.

It is thus possible to distinguish three types of sexual differentiation :

- (a) The gonad—ovary or testis.
- (b) The accessory reproductive organs.
- (c) The secondary characters.

The accessory reproductive organs in the male are all designed to convey the spermatozoa in a suitable medium to the exterior and thence into the female genitalia. The accessory organs of the female mammal (individually described in Chapter II) are adapted for reception of the spermatozoa, gestation of the

fertilized ovum, evacuation of the foetus, and subsequent suckling of the young.

The secondary sexual characters in mammals are extraordinarily diverse. The characters usually consist in the appearance or accentuation of some attribute which serves to attract the opposite sex, or combat others of the same sex. Since the male is usually the active partner in mammalian reproduction, this sex has the more definite secondary sexual characters. Thus the possession of fighting weapons and a stronger skeleton are typical of the male mammal. Sex differences in the voice and in the amount and distribution of hair are also found.

The differences in external appearance which result from the possession of secondary sexual characters make it possible in many animals to distinguish male from female without reference to their genitalia. The degree to which these characters are present, however, is subject to much specific variation, and from the point of view of the experimental physiologist, their distribution is disappointing. The common laboratory rodents, for instance, have practically no secondary sexual characters, and long experience is required to distinguish male from female in such animals as mice, rats, guinea-pigs, and rabbits without examination of the external reproductive organs.

*The mechanism of sexual differentiation.* The factors which cause a fertilized ovum to develop a testis or an ovary, namely, to become male or female, are at present inadequately known, but there can be little doubt that the development of the indifferent embryo into one sex or the other is normally dependent upon its chromosome constitution. This aspect of the problem has been dealt with in full by many writers (81, 147, 149, 168, 248).

The nature of the subsequent sexual differentiation is better understood, and it is clear that once the gonads have developed, differentiation proceeds as the result of stimuli from these organs. Gonadectomy experiments have shown decisively that the development of the accessory organs and secondary sexual characters is entirely dependent upon the presence of the gonad, as are the skeletal and other structural details typical of the two sexes.



Moreover, grafting experiments have shown that the removal of the gonad from its normal site in the body to an alien situation does not affect the development of the associated organs. Experiments of this nature soon suggested that the gonads exert their control by means of some internal secretion, and later work has substantiated this view. The extraction of the active principles from the gonads has, however, lagged behind work on the other internal secretory organs. This is particularly true of the testis, from which no substance appears to have been prepared capable of replacing its endocrine action in the castrated male. As regards the mammalian ovary, at least one definitely active substance has been prepared, and it is reasonable to suppose that both ovary and testis control the development of their accessory organs by means of internal secretions. In the female mammal the study of the endocrine activity of the gonad is complicated by the cyclic changes which take place in the reproductive organs during the breeding season, and which have no analogue in the male.

## CHAPTER II

### THE FEMALE REPRODUCTIVE ORGANS

THE morphology of the female reproductive organs is described in a great number of text-books and monographs (some of the chief of which are cited in the bibliography: 81, 265, 429) and it is not proposed to give a more detailed account here than is strictly necessary as a basis for the rest of the book. Morphological variation in the reproductive organs from one species to another may make one animal useless and another ideal for some particular experiment, and it is with this aspect of specific variation, therefore, that the present account is largely concerned.

#### (a) THE OVARY

The ovary is a bean-shaped organ attached to the broad ligament by the hilum. The outer covering is the germinal epithelium, which is continuous with the peritoneal epithelium, and from which the definitive ova are proliferated early in life and possibly after puberty (7, 275, 484). The body of the ovary consists of a stroma of connective tissue in which the follicles are embedded, together with the products of follicular degeneration or maturation. In addition, the ovaries of many animals (notably the rabbit) contain blocks of epithelial cells, forming the so-called interstitial tissue. This tissue appears to be completely absent in certain animals, such as the mouse.

*The Graafian follicle.* The follicle, containing the ovum, consists of two peripheral layers, the theca externa and the theca interna, surrounding the follicular epithelium (membrana granulosa and discus proligerus) which carries the ovum. In the more mature follicles (except in the Monotremata) an antrum appears which is filled with a viscous fluid, the liquor folliculi.

The theca externa cells are fibroblastic, and continue unchanged during the whole life of the follicle. After ovulation the theca externa produces trabeculæ which carry blood and lymph capillaries into the developing corpus luteum.

The accounts of the life history of the theca interna cells vary greatly. In some species, such as the pig (Corner, 121, 122),

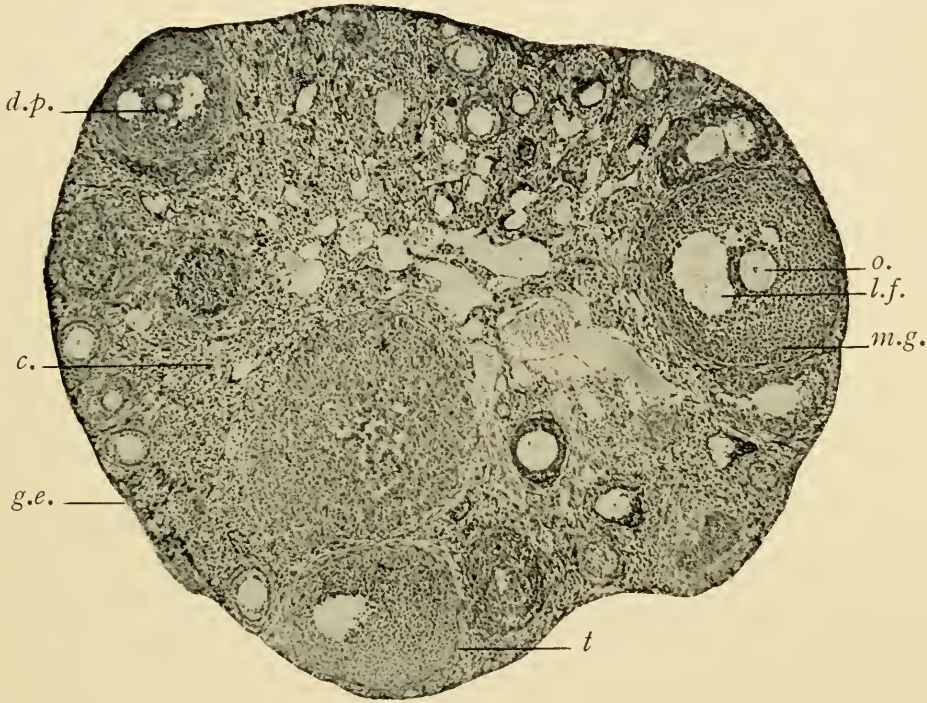


FIG. 1.—OVARY OF MOUSE JUST BEFORE PUBERTY.

Large follicles but no corpora lutea are present.

*c.* connective tissue; *d.p.* discus proligerus; *g.e.* germinal epithelium;  
*l.f.* liquor folliculi; *m.g.* membrana granulosa; *o.* ovum; *t.* theca.

and *Ornithorhynchus* (Hill and Gatenby, 299), they are described as being small and flattened until just before ovulation and then swelling up to a glandular type. Other authors, however, describe them as undergoing degeneration on follicular maturation or after ovulation (*Dasyurus*: O'Donoghue, 477, 479, 480; *Dasyurus* and *Didelphys*: Hill and Gatenby, 299).

The granulosa is made up of polygonal cells arranged irregularly, except round the periphery and round the ovum, where the arrangement is columnar. Most recent authors have followed Pflüger (517), de Winiwarter (639), and Waldeyer (628)

in supposing that both ovum and follicular epithelium are derived direct from the germinal epithelium. The ovum itself consists of an external zona pellucida which encloses a cytoplasmic mass containing the nucleus and certain cytoplasmic inclusions.

Ovulation, which takes place spontaneously at œstrus in most species, is preceded by certain changes in the follicle. The distension due to the accumulation of liquor folliculi causes the follicle to approach the periphery of the ovary, so that discharge of the ovum becomes possible. The discus proligerus, containing the ovum, breaks away from the membrana granulosa, and at the same time the cells become arranged radially round the ovum, from which they tend to withdraw, forming the corona radiata or cumulus oophorus. Finally, the first polar body is given off, and ovulation takes place. The nature of the final stimulus required for ovulation is not known. If fertilization takes place, the second polar body is given off, and the ovum proceeds down the Fallopian tube. Otherwise, degenerative fragmentation occurs, which has occasionally been mistaken for parthenogenesis.

Although ovulation is the predestined end of the Graafian follicle, the majority fail to complete the full life history owing to their excessive number, and undergo atresia at some stage of their growth. The degenerative processes usually begin in the granulosa, from which odd cells are shed into the antrum in a state of pycnosis. The degeneration of the ovum, accompanied by spurious maturation divisions, usually takes place later in atresia. Finally, the follicle is either entirely absorbed or metamorphosed into interstitial tissue or a corpus luteum atreticum. Cyclic bursts of atresia seem to take place in the ovary corresponding with the stages of the œstrous cycle. Atresia of small follicles tends to be most common during pregnancy and in the vicinity of large corpora lutea. Atresia of large follicles is very evident in the rabbit (in the absence of the sexual stimulus required for ovulation) and in the guinea-pig where only two or three of each batch of mature follicles ovulate. For the extensive literature on follicular atresia the bibliography given by Salazar (541) may be consulted.

Abnormalities of the ovum and follicle, such as multinucleate



ova, and poly- or anovular follicles, are not uncommon, but they are of no particular physiological interest (see Hartman, 274, for full bibliography).

*The corpus luteum.* After ovulation the shell of the ruptured follicle shrinks, and, owing to the previous rupture of capillaries, becomes filled with a greater or lesser amount of blood. From



FIG. 2.—OVARY OF DOG.

*a.f.* atretic follicle; *c.l.* corpus luteum; *y.g.f.* young Graafian follicle.

the follicular remains, the corpus luteum, composed of large glandular cells containing the so-called lutein granules, develops with remarkable rapidity. In some species (*e.g.* cow, man) the corpora lutea are coloured yellow, orange or reddish, by the presence of carotene.

Much controversy centres round the exact manner of formation of the corpus luteum. The probability is that it develops from the remains of the follicle by enlargement of the individual cells and not by cell division, though Loeb (379) has described

mitotic proliferation. Doubt still exists as to the degree to which the various follicular elements are concerned in the transformation.

Von Baer (53) originally described the corpus luteum as originating solely from the theca interna, and one or two comparatively recent writers have adopted this view. It is now generally admitted, however, that the remains of the

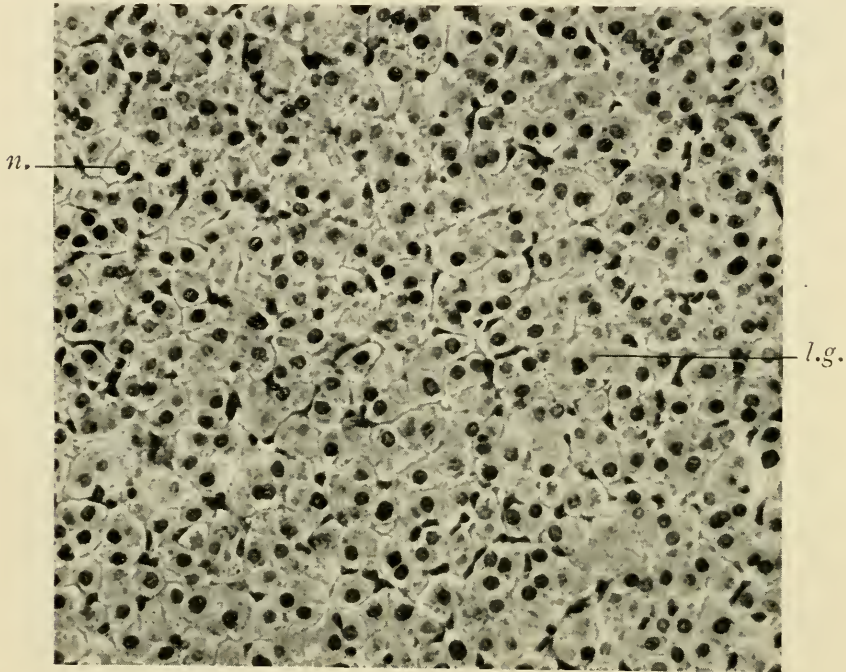


FIG. 3.—PART OF CORPUS LUTEUM OF PREGNANCY OF MOUSE.

*l.g.* lutein granule; *n.* nucleus.

follicular epithelium play the major part in the formation of the true lutein cells, and the real problem relates to the part played by the theca interna. According to Sobotta for the mouse (583-6) and guinea-pig (587), Van der Stricht for the bat (601-3), and Marshall for the sheep (440, 442), the lutein cells are derived exclusively from the follicular epithelium, the theca interna merely assisting in supplying the vascular connective tissue framework for the corpus luteum.

In other species, however, the theca interna has been described as undergoing a burst of growth at the time of ovulation, and



as contributing clumps of cells to the corpus luteum. These cells, the theca-lutein cells of Corner and para-lutein cells of Gatenby, while clearly distinguishable from the true lutein cells, are of the same general type and appear to be definitely of a secretory nature. This type of corpus luteum has been described in the sow by Corner (122), in Platypus by Hill and Gatenby (299) and in the human by Gatenby (237) and by Shaw (560).

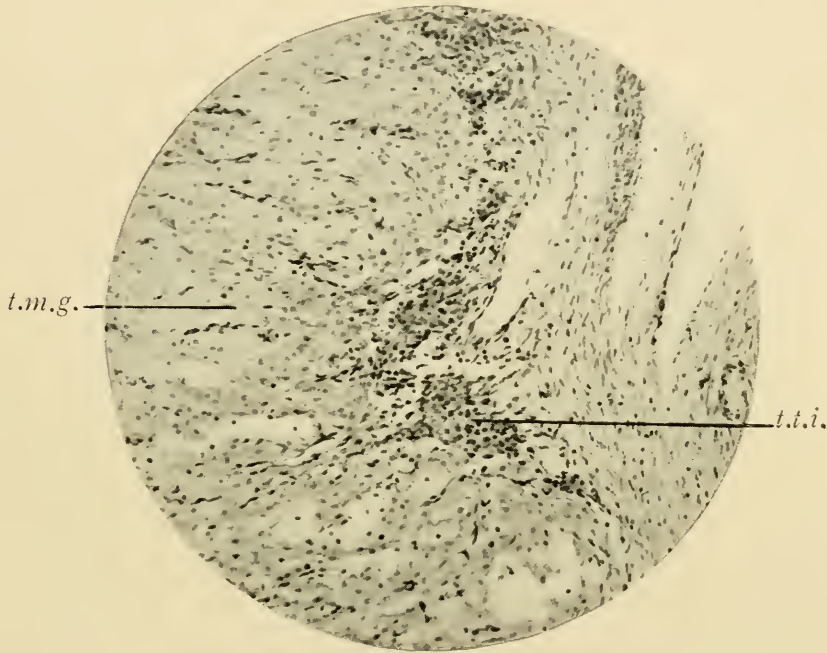


FIG. 4.—HUMAN CORPUS LUTEUM.

*t.m.g.* tissue derived from membrana granulosa; *t.t.i.* tissue derived from theca interna (after Shaw).

In such circumstances, therefore, there is some histological basis for supposing the corpus luteum to have a dual secretion.

In certain animals, notably the cow, many of the corpora lutea are hollow and contain a viscous fluid. Some workers have considered them to be cystic. Allen in the mouse (6) and Hammond in the cow (265), however, found that the secretion of liquor folliculi goes on temporarily after ovulation, and if not completely reabsorbed by the developing corpus luteum, the fluid remains in a central cavity. This inclusion of follicular secretion is important from the point of view of extraction of the corpus luteum (see p. 107).

In many species, notably polyœstrous ones, the corpus luteum survives as a histologically intact body long after it must have ceased to function; in the unmated mouse three, four or even five sets of corpora lutea, representing successive ovulations, may be present in the ovary at one time. The name corpus luteum spurium has been given to these corpora lutea of the infertile cycle, but the distinction seems unnecessary since the difference between them and the corpora lutea of true pregnancy



FIG. 5.—HUMAN OVARY.

*n.c.l.* new corpus luteum; *o.c.a.* old corpus albicans (after Shaw).

is merely one of degree. In *Dasyurus* such a difference has not appeared, the duration of life and histological appearance of the corpora lutea being uninfluenced by the fate of the ova. The ferret appears to be similar in this respect.

The corpora lutea atretica, which are sometimes formed without the intermediate stage of ovulation by atretic follicles or by follicles in which ovulation is for some reason inhibited, probably do not function; here again, however, the difference from normal luteal tissue appears to be only one of degree. Corpora lutea capable of performing all their known functions can be produced experimentally without discharge of the ovum.



After a certain time, which varies according to the species and the occurrence of lactation and pregnancy, the corpus luteum retrogresses and finally is either reabsorbed entirely or dwindles to a small corpus albicans.

*The interstitial tissue of the ovary.* The whole question of the presence, formation and function of the so-called interstitial tissue of the ovary is controversial. Clumps of large epithelial

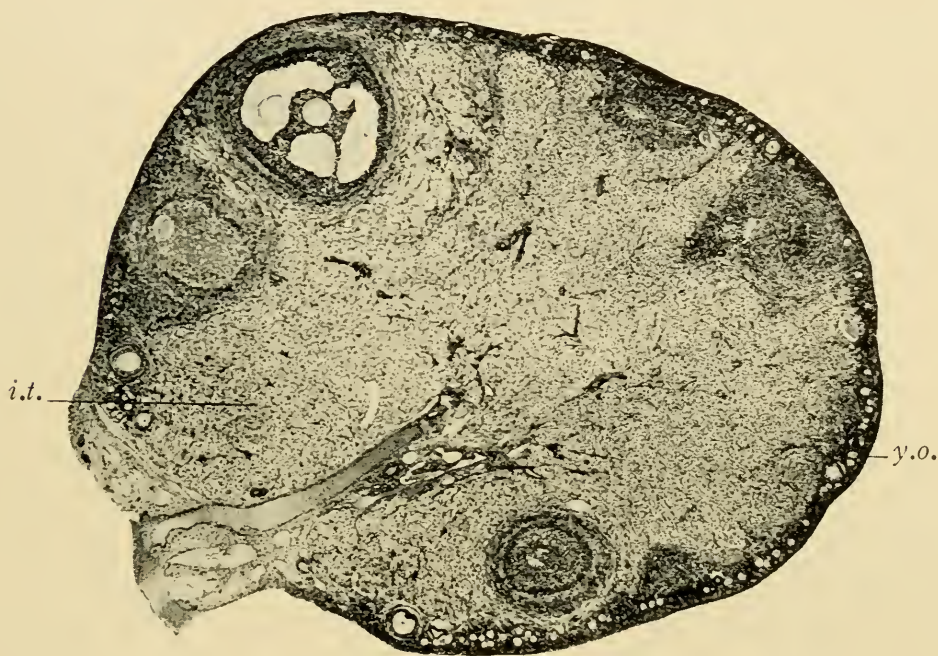


FIG. 6.—OVARY OF RABBIT.

*i.t.* interstitial tissue; *y.o.* young oocytes.

cells, presenting the appearance of secretory tissue, are very obvious in the ovaries of such mammals as the rabbit, but appear to be absent in others such as the mouse. Schaeffer (545), Aimé (3), Fraenkel (210), and O'Donoghue (480) give lists of species showing the presence or absence of interstitial cells. According to Bouin and Ancel (77) they are not found in the ovaries of animals which ovulate spontaneously. The confusion as to the distribution of this tissue is accentuated by the lack of any unanimous definition. Some workers describe only obviously extrafollicular tissue as interstitial, while others, maintaining that interstitial cells are of follicular origin, apply the term to the

products of follicular atresia and luteal degeneration. Their origin is variously ascribed to three different sources :

- (a) Direct derivation from the germinal epithelium; Paladino (483), Lane-Claypon (340) and others.
- (b) Derivation from the follicle, either from old corpora lutea, atretic follicles or theca interna; Schron (550) and Rabl (522).
- (c) Derivation from transformed connective tissue cells; Sainmont (540), Regaud and Policard (527) and Athias (47).

Others again, as Van der Stricht (603), consider a combination of one or more of these sources to be probable. Criticisms of all these views are obvious and further discussion is unwarranted here.

The interstitial tissue has been said to show cyclic changes during the œstrous cycle (O'Donoghue, 480, Lane-Claypon, 340), but owing to the present doubt as to whether it is an essential constituent of the mammalian ovary, and to the uncertainty of its origin, it is difficult at the moment to assign to it any definite physiological rôle. According to Steinach (590), Lipschütz (366) and others, it is the main endocrine tissue of the ovary and constitutes the 'puberty gland' of these authors.

*Accessory ovarian tissue.* Ovarian tissue apart from the two main ovaries may occur in rare instances, according to Waldeyer (628), Beigel (55), Williams (638), Hartman (276) and other workers. In the dissection of some thousands of mice, however, the writer has found only two with accessory ovaries, and in each case the abnormality consisted of a supplementary body within the same capsule as the normal ovary. The problem of third or accessory ovaries is of physiological importance as it may complicate the operation of complete ovariectomy (see p. 98).

## (b) THE ACCESSORY REPRODUCTIVE ORGANS

In mammals the accessory female reproductive organs are designed for the fertilization of the ovum, the gestation of the embryo, and the subsequent suckling of the young. To this end

the more primitive oviduct of oviparous animals has undergone great elaboration, while certain skin glands have undergone alteration to produce the mammary tissue.

*The Fallopian tube.* After ovulation the ova are caught by the funnel-shaped end of the Fallopian tube (the infundibulum) and are passed down the tube by the action of the ciliated epithelium with which it is lined, aided by the secretion of mucus which takes place at the time of ovulation. The Fallopian tube may

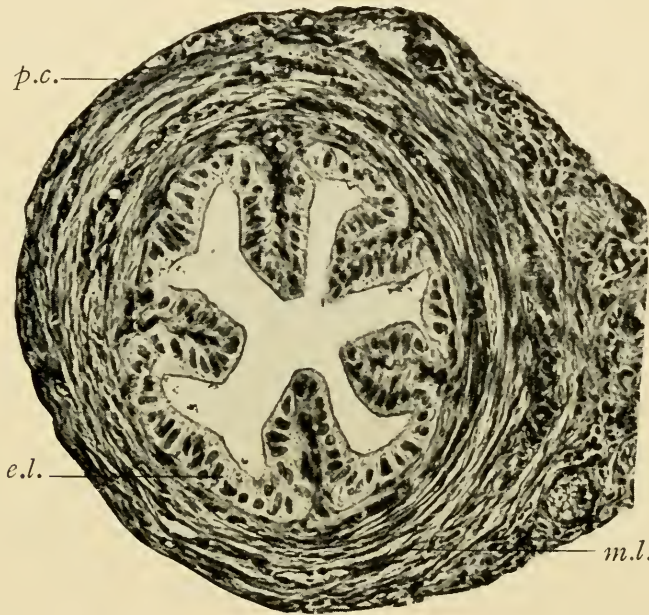


FIG. 7.—FALLOPIAN TUBE OF MOUSE.

*e.l.* epithelial lining; *m.l.* muscle layer; *p.c.* peritoneal covering.

be entirely independent of the ovary except for its common anchorage in the broad ligament (as in man, the cow, sheep, etc.) or the expanded end may open into an ovarian capsule formed from a fold of peritoneum. A closed circuit of this nature, which makes it impossible for the ova to fall into the body cavity, is found in the bitch, mouse, and rat. The Fallopian tube itself may be short and coiled as in the mouse and rat, or long and comparatively straight as in the rabbit, ewe and man. The tube is lined by mucous membrane and covered by a serous layer from the peritoneum. Circular and longitudinal muscle layers are found under the serous layer.



*The uterus.* The Fallopian tubes open into the uterus, which consists essentially of the same three layers, but the internal mucous membrane is much thickened to form the uterine mucosa (or endometrium) which consists of a glandular stroma lined by epithelium. The shape of the uterus shows great specific variation. At one extreme is the type having two distinct cornua fusing only at the junction with the vagina, and even there retaining two distinct cervical canals. This type of uterus is characteristic of the rat and the mouse. At the other extreme is the type where the cornua are entirely fused to form one large (usually pear-shaped) uterine sack, into the top angles of which open the two Fallopian tubes. This type is characteristic of the human. Between these extremes every gradation is found, from the rabbit, where the cornua are fused to the extent of having a common cervical canal, to the goat where fusion is complete except for the tops of the cornua, which form two horns projecting from the main body of the uterus.

*The vagina.* The vagina, which connects the uterus with the exterior, possesses the two muscular layers found in the other sections of the genital tract, and is lined internally with epithelium, the nature of which varies greatly in different animals and in different stages of the œstrous cycle. In man erectile tissue is present, but this appears to be absent in the lower species, in which the necessary facilitation to copulation is obtained by copious secretion (cow), intense hyperæmia (ferret) or cornification (mouse). The vagina opens to the exterior at the vulva, the anatomy of which varies greatly in different species, being a simple orifice in the case of most lower mammals, and complicated in the human by inner and outer labia.

In the rat and mouse the vaginal lumen is not complete during pre-pubertal life. In the foetus the cord of cells destined to form the vagina first shows a lumen at the anterior end. This lumen extends until it is separated from the exterior only by a thin wall of cells, which remains in the rat and mouse until the first œstrous period, when it is ruptured by the enlargement of the vagina. The closure of the immature rodent vagina may be analogous to the partial closure of the human vagina effected

by the hymen. In the guinea-pig a remarkable mechanism exists whereby the vaginal closure membrane is regenerated after each œstrous period.

*The clitoris.* The clitoris, situated anteriorly to the vaginal orifice, is a vestigial homologue of the penis. It is composed of connective tissue, surrounded by more or less cornified epithelium. Its homology with the penis is emphasized by the presence (in the human) of erectile tissue; in such lower

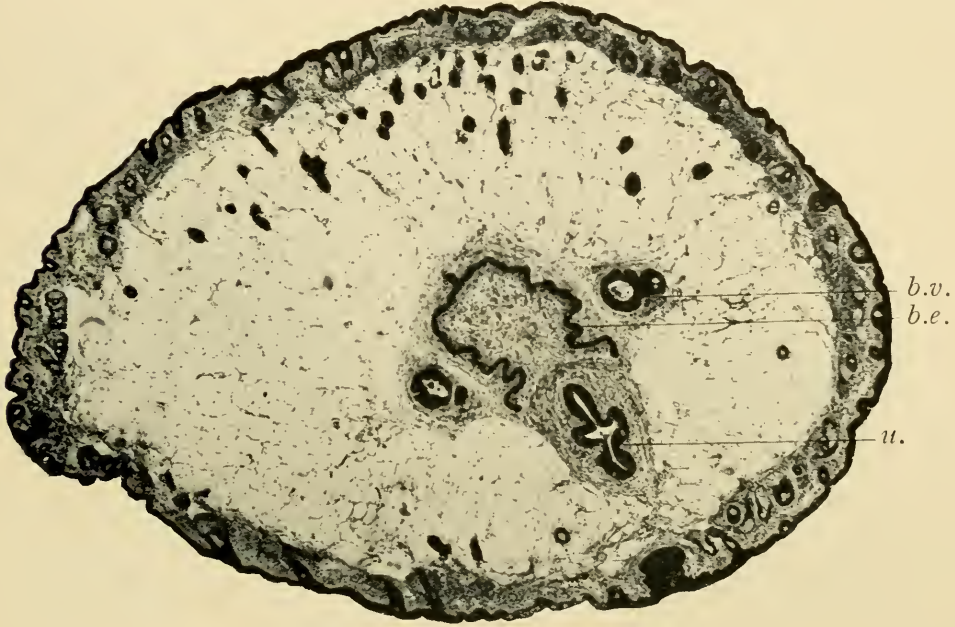


FIG. 8.—CLITORIS OF MOUSE.

*b.e.* band of epithelium running down from distal cleft; *b.v.* blood vessels; *u.* urethra.

mammals as the rat and mouse, the urethra, instead of opening to the exterior at the vulva, as is usually the case, traverses the clitoris and emerges at its distal cleft. In certain mammals the clitoris plays a subsidiary part in copulation, but otherwise its value in the reproductive processes appears to be negligible.

*The mammary glands.* The mammae are usually bilaterally paired organs, consisting of secretory alveoli from which the milk is carried to the exterior by ducts. The ducts are gathered together on the surface into the nipple to facilitate suckling. The exact nature of the secretion which takes place in the mammae is still a subject of controversy, but, whether or not

actual cell destruction is involved, it seems probable that the secretion is the manifestation of a katabolic phase resulting from withdrawal of a growth stimulus.

The number of mammæ and their distribution varies widely in different species. The smallest normal number appears to be one pair, while in the larger polytocous animals (pig), as many as six to nine pairs may be found. The rat, rabbit and mouse have five to six pairs. Where there are only

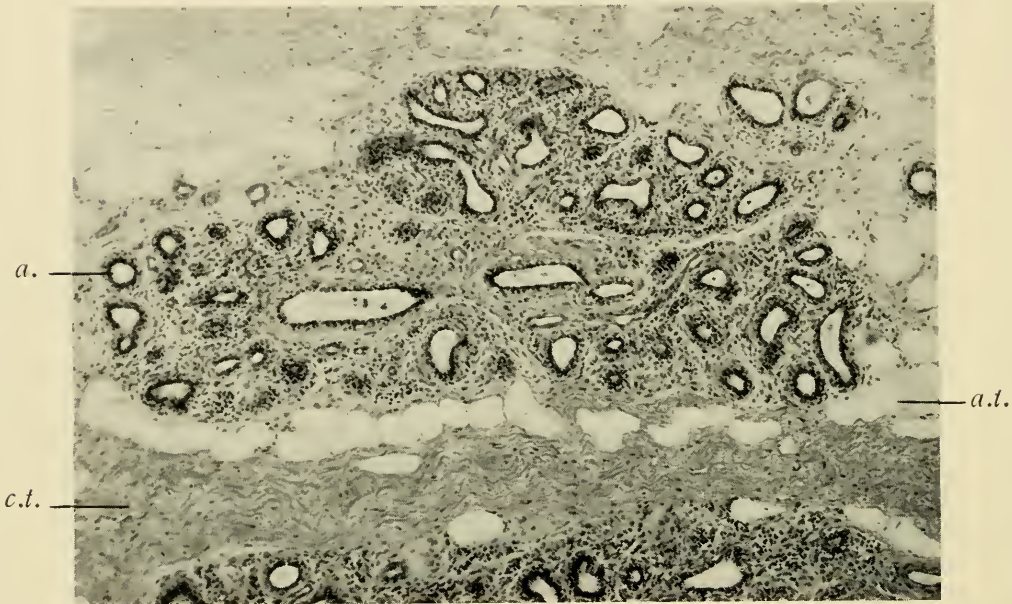


FIG. 9.—LOBULE OF MAMMARY GLAND OF COW.

*a.* alveolus; *a.t.* adipose tissue; *c.t.* connective tissue.

one or two pairs they may be either thoracic (Primates, elephant) or abdominal (guinea-pig, cow, etc.). The presence of a large number of mammæ involves their distribution over both thorax and abdomen. In certain animals the mammæ have milk reservoirs, which involve alterations in the shape of the gland, resulting in the udder typical of ruminants. The rat, mouse, rabbit, and ferret, on the other hand, have no receptacles for the milk beyond the ducts, which may become greatly distended; the glands in these animals are flat strips of tissue beneath the skin. In the rabbit the gland can readily be stripped off from both skin and body wall and can be prepared



entire for examination with great ease, a fact which makes this animal invaluable for experimental work. The mammary



FIG. 10.—UDDER OF COW; STAINED TO SHOW MAMMARY TISSUE.  
(From Hammond).

gland differs from the other accessory organs in being differentiated at a much later stage of development and also in being rudimentary when puberty is reached.



## CHAPTER III

### SEXUAL PERIODICITY IN THE FEMALE MAMMAL

SEXUAL periodicity in the mammalian female may be said to consist of three cycles. The first—the attainment of puberty, sexual maturity, and the decline of sexual function at the menopause—is passed through but once. The second—the periodic occurrence of the breeding season—appears one or more times each year (or in extreme cases every two years). Finally, the third consists of the cyclic periods of œstrus, at which the actual mating takes place, and of which one or more occur during each breeding season.

#### (a) PUBERTY AND THE MENOPAUSE

The development of the ovary before puberty tends to be sporadic, and may include one or more waves of growth followed by retrogressive changes. In the mouse, for instance, the growth of the follicles is quite advanced at three weeks old, but degeneration subsequently sets in, accompanied by a decrease in the size of the ovary (80). The growth of the accessory organs, on the contrary, appears to be gradual and continuous from the time when they are first differentiated. The first abrupt change in the accessory organs occurs at the first œstrous period, the definite sign of the onset of puberty. The attainment of puberty may thus be said to consist of two phases:

- (a) The gradual pre-pubertal development of the accessory organs.
- (b) The abrupt appearance of the first œstrus and ovulation.

The first œstrous period, however, is the same as any other, except for minor changes such as the appearance of the vaginal orifice for the first time in the mouse and rat; the stimulus



FIG. 11.—OVARY OF MOUSE AT THREE WEEKS OLD, SHOWING  
LARGE NUMBER OF SMALL FOLLICLES.

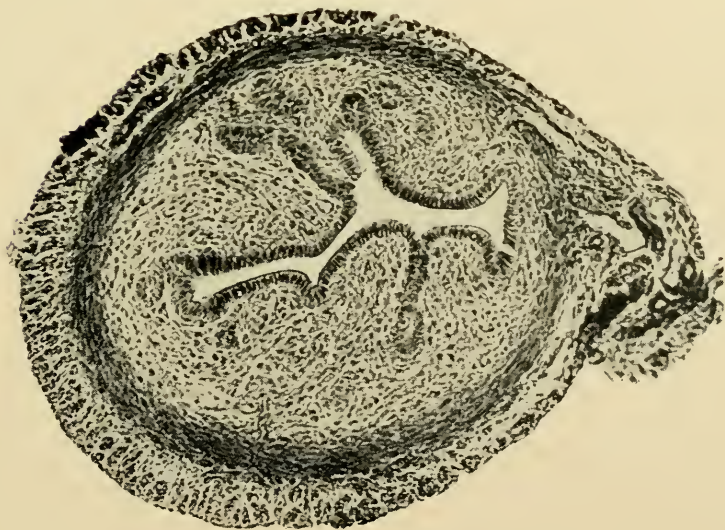


FIG. 12.—UTERUS OF MOUSE AT THREE WEEKS OLD, SHOWING  
GENERAL LACK OF DEVELOPMENT.

causing the first œstrous period must be supposed to be the same as that responsible for the later ones. The problem of the causation of puberty thus resolves itself into two parts:

- (a) What stimuli cause the gradual pre-pubertal development of the accessory organs?
- (b) By what means is the œstrus-producing stimulus first set in motion?

These questions are discussed later (see p. 123).

Functional puberty is attained in many animals (notably in man) before body growth is completed. This is an anomaly, since animals becoming pregnant before growth is completed rarely reach maximum size. In the rat, according to Long and Evans (425), the vaginal orifice usually appears a little before the first ovulation. In the mouse and the rat it has been observed that the intervals between the first few œstrous periods tend to be somewhat longer than normal (425, 491).

Long and Evans give the following data for the attainment of puberty in the rat:

TABLE I.—ATTAINMENT OF PUBERTY IN THE RAT  
(after Long and Evans).

	Average.
Age at opening of vagina -	72nd day of life
Age at first ovulation - -	77th day of life
Length of 1st cycle - -	10 days
Length of 2nd cycle - -	9 days
Length of 3rd cycle - -	8.5 days
Length of 4th cycle - -	7.3 days

On the whole, however, the remarkable thing about the onset of puberty is that the normal periodic activity is attained so abruptly.

The decline of sexual function at the menopause is characterized by retrogressive changes in the ovaries and by the gradual atrophy of the entire accessory sexual apparatus—Fallopian tubes, uterus, vagina, vulva, and mammary glands. These changes, which finally are similar to those occurring after



ovariectomy, have been described, especially for the human, by many writers.

The fading out of the sexual cycle at the menopause is far less abrupt and regular than is its appearance at puberty. In the human, the menopause extends over two or three years, menstruation gradually becoming less frequent and less regular. According to Magian (433) the cessation of menstruation does



FIG. 13.—OVARY OF SENILE MOUSE 15 MONTHS OLD, SHOWING LACK OF FOLLICLES AND NEW CORPORA LUTEA (*cf.* fig. 1).

*o.c.l.* old corpus luteum; *s.a.f.* small anovular follicle.

not necessarily involve the complete disappearance of fertility, and the ovarian cycle may therefore go on longer than the uterine.

Data relating to the menopause in lower mammals are very difficult to obtain. Domesticated animals are usually slaughtered when the breeding function declines, and in laboratory animals the decreased resistance to disease which occurs in old age usually results in death about the time when menopause symptoms appear. Nevertheless some data are available.

In the mouse the decline of the sexual function usually proceeds by the following stages:

- (a) Litter size gradually decreases.
- (b) Coitus becomes infertile owing to ovulation having ceased.
- (c) Œstrous symptoms in the accessory organs become infrequent and irregular.
- (d) Complete menopause anœstrus appears.

By the last stage the ovary has become entirely devoid of follicles as well as of corpora lutea vera and resembles to a great extent the type produced by exposure to X-rays (see p. 138).

#### (b) THE BREEDING SEASON

The term 'breeding season' was originally proposed by Heape (287) to cover the time when activity occurs in the reproductive organs. This definition, however, includes the period of pregnancy and lactation, and since some mammals may spend all their reproductive life in one or other of these states, the term loses some of its force. In discussing here the time of the year at which breeding takes place, the term 'breeding season' will be used to denote the time at which a species comes into œstrus, namely, in the sense that 'sexual season' was used by Heape.

In captive and domesticated mammals, as well as in man, living under conditions of fairly constant food supply (and often of temperature), the occurrence of a restricted breeding season has become rare. The lower mammals in a state of nature, however, have a definite season of the year at which mating takes place, and, in general, this season is so placed that the young are produced at an auspicious time. Copulation takes place, even during the breeding season, only at certain definite periods of œstrus (or 'heat'). Œstrus may occur only once in a breeding season (monœstrous animal) or, in the absence of pregnancy, a regular series of periods may occur (polyœstrous animal). During the non-breeding season (except in pregnant animals) the reproductive organs are in a state of quiescence. This period is known as anœstrus in contrast to œstrus.

The breeding season is well shown in wild rodents, where a

series of œstrous periods, probably separated by pregnancy, occur during the spring and summer, while during the winter months the reproductive organs enter into a prolonged state of rest. In animals with a long period of gestation, what would be the anœstrous period may be occupied by pregnancy. Mares, for instance, having a period of gestation of 11 months, foal about the beginning of the next breeding season.

The wild prototypes of the cow, pig, sheep and goat all probably exhibit a restricted mating season, which in the case of the last two appears to be in the autumn, but domestication has resulted in the gradual expansion of the season until the domestic strains of these animals will breed at almost any period of the year. The greatest readiness to breed, however, is still found at a time which probably corresponds to the primitive breeding season, especially in the less highly domesticated varieties.

The dog has two breeding seasons a year, in spring and autumn, and only one œstrous period occurs in each season. Whatever the primitive state of affairs may have been, the monœstrous cycle, with its limited opportunities for the animal to become pregnant, is the rarer condition at the present time in such animals as have been studied. The cause of the onset of the breeding season of mammals is obscure, but, in so far as it occurs in spring and summer, it is probably a combined effect of raised temperature and increased food supply (359, 509).

Civilized man retains practically no vestiges of a breeding season beyond a slight seasonal variation in the birth rate (especially the illegitimate birth rate), and therefore in the conception rate. Many primitive tribes, however, show a marked lumping of the births in one season of the year (444), and it is possible that man originally had a definite, if not entirely restricted, breeding season.

### (c) ESSENTIAL FEATURES OF THE ŒSTROUS CYCLE

The essential feature of ovarian activity is the maturation of the Graafian follicle and the discharge of the ovum. This periodic occurrence, together with the intervening growth of the corpora lutea, constitutes the ovarian cycle. While these



ovarian changes are in progress cyclic events are proceeding in the accessory organs of reproduction—uterus, vagina, and mammary glands. These events are collectively known as the ‘œstrous cycle,’ from œstrus, the central point of the cycle, when ovulation and copulation take place.

After the anœstrous or pre-pubertal period of comparative quiescence the cycle starts with the preliminary phase of proœstrus, during which the follicles ripen and growth changes take place in the accessory organs. This is followed by the period of œstrus proper, during which ovulation takes place (in some species only after copulation), accompanied by further changes, usually of a retrogressive nature, in the accessory organs. It is at this period only, in the lower mammals, that the female will receive the male (but see p. 55). Œstrus is usually followed by a short recuperative period—the metœstrus; subsequent events depend primarily on the fate of the ova produced at œstrus and on the species of animal.

In the absence of pregnancy, the monœstrous animal with only one œstrus per breeding season, returns to anœstrus, usually with an intervening period of development in the ovary and accessory organs. The polyœstrous animal, on the other hand, with a series of cycles in a breeding season, enters upon a very transitory period of development, the diœstrous interval. At the end of this short phase proœstrus supervenes and the cycle starts again. The occurrence of successive œstrous periods with no real interval of rest constitutes a diœstrous cycle, of which the essential feature is that growing follicles or corpora lutea are present the whole time in the ovary.

After ovulation, conditions in the non-pregnant female are determined by the behaviour of the corpus luteum. This may show only the most transitory development, as in the rat and mouse, or, as in Marsupials and the ferret, it may undergo development equal or nearly equal to that found during pregnancy, with very striking growth effects upon the accessory organs. In the latter case the term ‘pseudo-pregnancy’<sup>1</sup> is

<sup>1</sup> This term, coined by Matthews Duncan (172) to indicate a psychological condition in the human female, was applied to the post-ovulation phase in *Dasyurus* by Hill and O’Donoghue (300), and later to the same phase in the bitch by Marshall and Halnan (449).

given to this period of post-ovulative activity. The two chief types of cycle may therefore be illustrated as follows:

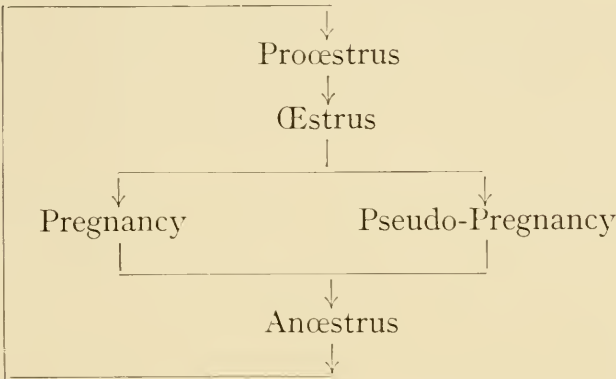
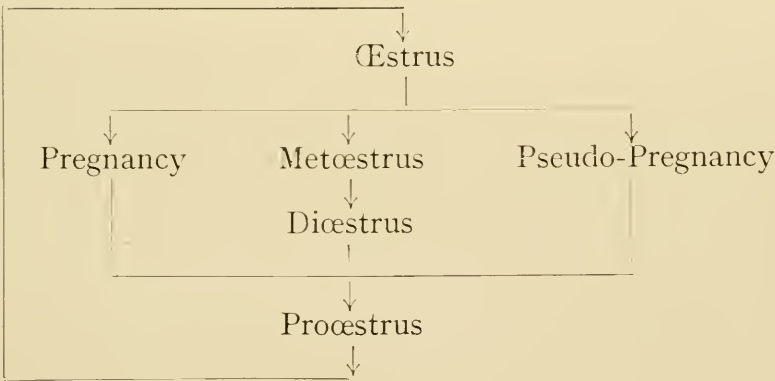


DIAGRAM TO ILLUSTRATE MONESTROUS CYCLE.

One œstrous period per breeding season. A complete cycle occupies the whole breeding season, which is separated from the next by anœstrus (as found in dog).



SINGLE DIENTROUS CYCLE, WHICH IS REPEATED A NUMBER OF TIMES DURING THE BREEDING SEASON.

In the rat and the mouse pseudo-pregnancy is dependent upon sterile copulation, while in the guinea-pig diœstrus is really pseudo-pregnancy (see p. 35).

The general account given above has assumed ovulation to be spontaneous, *i.e.* to occur automatically at œstrus irrespective of copulation. In two common mammals, however, the rabbit and ferret, ovulation occurs only after copulation. In the absence of the male, œstrus, including the presence of mature follicles in the ovary, persists right through the breeding season, at the end of which follicular atresia takes place. In these

two animals, therefore, no cycle exists in the unmated animal, except the alternation of anæstrus and breeding season.

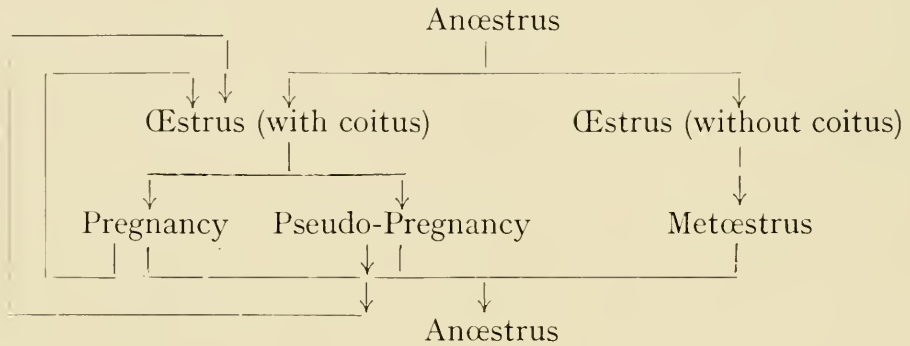


DIAGRAM OF CYCLE WHERE OVULATION IS DEPENDENT ON COPULATION, AS IN THE RABBIT AND FERRET.

As has been pointed out above, cyclic changes occur in the secondary organs in correlation with those in the ovary. The extent to which the various accessory organs participate in the œstrous cycle shows much specific variation. All species which have been studied show considerable uterine changes, but vaginal and mammary cycles have been less frequently described. In the ferret, guinea-pig, rat, and mouse, however, vaginal changes at least as marked as the uterine ones are well known, while cyclic mammary changes in the unmated female have been described in the rat (472, 605), guinea-pig (402), cow (265), and human (159); in other species, apparently, they may be absent. The extent to which the vagina participates in the cycle entirely determines the application to any particular species of the vaginal smear technique (see p. 95).

The exact nature of the changes taking place in the accessory organs varies very much from animal to animal, but they are roughly comparable; a comparison of the ovarian and uterine cycles is given on p. 27.

For descriptive purposes it is convenient to use Loeb's (400) division of the cycle into two main phases: (a) the follicular phase, during which the Graafian follicles mature and ovulate (this includes proœstrus and œstrus) and (b) the luteal phase (including dioœstrus, pseudo-pregnancy and pregnancy) during which the corpus luteum dominates ovarian activity.

Phase	Ovarian cycle	Uterine cycle (and vaginal cycle where occurring)
Anœstrus	Rest	Rest
Proœstrus	Maturation of follicles	Growth
œstrus	Ovulation	Degeneration Copulation
Pseudo-Pregnancy	Development of corpus luteum	Extensive pseudo-pregnant development
Anœstrus	Rest	Rest
Metœstrus	Formation of corpus luteum	Recuperation
Diœstrus	Transitory development of corpus luteum	Transitory or no development
Proœstrus	Maturation of follicles	Growth

The diversity in the details of the cyclic changes in the female genitalia makes any sort of classification of the types of cycle extremely difficult. Scarcely two mammals, except the rat and mouse, are entirely comparable, and species which are closely related zoologically may show entirely different features in the sexual cycle. The rat and the rabbit, for instance, are extraordinarily different in their reproductive phases. The division of animals into monœstrous and polyœstrous is unsatisfactory from a descriptive point of view because (*a*) the condition varies under domestication, and (*b*) the only common true monœstrous Eutherian is the dog.

Certain animals, however, have various salient points in common. The rabbit and ferret, for instance, both of which remain on œstrus throughout the breeding season in the absence of copulation, and do not ovulate spontaneously, are comparable to some extent. In the description of the œstrous cycles of the chief mammals, comparable species are as far as possible discussed together.

## CHAPTER IV

### TYPES OF ŒSTROUS CYCLE

#### (a) MARSUPIALS

*Dasyurus*. In *Dasyurus viverrinus*, studied by Hill and O'Donoghue (300), O'Donoghue (476), and Sandes (544), the anœstrous period lasts more than half the year. This is terminated by the onset of the active phase of the reproductive cycle, which is divided by Hill and O'Donoghue into four stages—proœstrus, œstrus, postœstrus, and pregnancy or pseudo-pregnancy. During proœstrus, which lasts from four to twelve days, characteristic changes occur both in the uterus and in the external genitalia. The marsupial pouch, for instance, enlarges, but according to O'Donoghue no mammary changes occur during œstrus. The uterine mucosa increases in thickness and becomes very vascular, the glands lengthen and become convoluted, while the epithelium tends to thicken. These processes are continued during œstrus, which lasts only for one or two days, and at which time copulation takes place. Ovulation is delayed until the next stage, to which Hill and O'Donoghue gave the name of 'postœstrus'. The length of this phase appears to be very variable, but the authors state that ovulation does not occur until five or six days after the end of œstrus. Ovulation is spontaneous and is remarkable because of the large number of ova liberated. Pregnancy lasts not less than eight and not more than fourteen days. In the absence of conception, changes occur which are essentially the same, and of the same duration, as those during pregnancy. Corpora lutea, indistinguishable from those of pregnancy, are formed in the ovary (544). Typical changes in the mammary glands, the uterus, and the marsupial pouch also occur. As a result of a detailed study of the growth changes in the mammary apparatus, O'Donoghue came to the conclusion that no difference is observable



between the development of pregnancy and of pseudo-pregnancy.

*The Opossum.* The opossum was originally thought to be monœstrous, like *Dasyurus*, but Hartman's (271, 278) detailed study of the cycle shows it to be polyœstrous. The diœstrous cycle in the opossum is, however, complicated by a

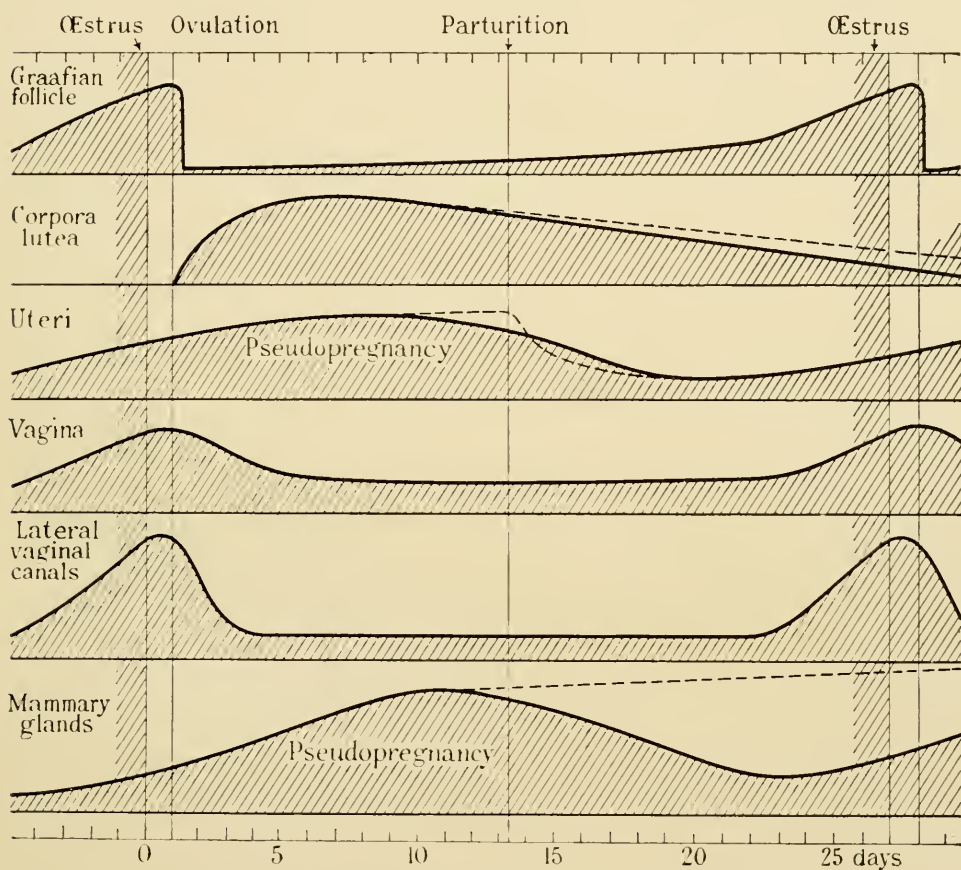


FIG. 14.—DIAGRAM OF ŒSTROUS CYCLE IN THE OPOSSUM.  
(From Hartman).

definite period of pseudo-pregnancy which, as a rule, is not well marked in polyœstrous animals. The recent work on the opossum has been greatly facilitated by the fact that this Marsupial, like the rat, mouse, and guinea-pig, shows characteristic œstrous changes in the vagina. With the aid of a vaginal smear technique, Hartman has been able to study in detail the various phases of the cycle, the total length of which is about twenty-eight days. He points out, however, that its duration may be

disturbed by follicular atresia. The opossum is remarkable among polyœstrous animals for the fact that pregnancy, which lasts only about thirteen days, occurs without disturbing the normal periodicity of œstrus.

The ovarian cycle consists of the rapid growth of Graafian follicles just before œstrus, and ovulation about one day later. The uterine cycle is characterized by growth at œstrus, which is continued during the pseudo-pregnant period or during pregnancy. During anœstrus the uterus shows a reduction of both the mucosa and the muscular layer. The glands are straight or only very slightly coiled, while the lumen is small and the epithelium has one layer. At proœstrus, when the Graafian follicles have attained about a quarter of their maximum size, the uterus undergoes distinct changes. The gross size becomes greater owing to increase in the various elements, to increased vascularity, and, above all, to infiltration of lymph into the mucosa. These changes begin in early proœstrus and culminate some days after œstrus. By the time that œstrus sets in, the uterus is considerably swollen and the glands greatly coiled. The lumen has become much enlarged. After ovulation the growth of the uterus continues, and is characterized by further hypertrophy of the mucosa and glands. At about the eleventh day after ovulation, in the absence of pregnancy, the uterus undergoes atrophy. The mucosa collapses owing to the withdrawal of lymph, and the epithelial glands undergo degeneration. The uterus then returns to the diœstrous condition in which the epithelial glands consist of low columnar or cuboidal cells, and degenerating material is found in the lumen.

The vaginal cycle shows equally obvious changes. During anœstrus, the epithelial lining is thin and there is no cellular debris in the lumen. At proœstrus the vagina grows in diameter and the epithelium becomes thickened. At the end of this stage the mucosa is at least twelve to fifteen cells thick. No leucocytes are to be seen, and the epithelium and the vaginal smear consist of large, flat, nucleated cells. At œstrus these nucleated cells are replaced in the vaginal smear by true cornified cells which arise from the cornified mucosa of the vagina. After ovulation, leucocytes begin to appear in the vaginal smear, and within three or four days the smear



consists of leucocytes with only a few cornified cells. Immediately after this, nucleated epithelial cells appear again. During diœstrus the vaginal smear consists of nucleated epithelial cells with a number of leucocytes. The opossum is remarkable in having lateral vaginal canals which undergo a similar cycle.

The mammary glands of the opossum have a very clear cycle. Growth begins at proœstrus, and continues uniformly until the end of pseudo-pregnancy or the end of true pregnancy. It is necessary to suppose that the beginning of this growth is under the control of the œstrus-promoting stimulus and that later the corpora lutea are responsible. The growth occurring during proœstrus is, however, comparatively slight compared with that found during pseudo-pregnancy. At the end of pseudo-pregnancy or true pregnancy, atrophy sets in, and the lowest point of development is reached about twenty-three days after the previous ovulation or some five days before the next ovulation is due.

(b) DOG

Some observations on the ovary of the dog were made by Bischoff (66) as early as 1845. Bouin and Ancel (75) and Van der Stricht (602) later studied the ovarian cycle. Friedlaender (233) appears to have been the first to deal with the uterine mucosa, while Retterer (533) and Keller (319) published more detailed work. The whole subject of the œstrous cycle in the dog has been investigated in detail by Marshall and Jolly (450), Marshall and Halnan (449), and more recently by Gerlinger (240, 241) and Evans and Cole (185).

With the exception of the vagina, the cyclic changes in the reproductive organs are very definite. During anœstrus, the ovary is small and contains neither large follicles nor functional corpora lutea. Primordial follicles are, however, developing. The uterus is thin and anaemic. The mucosa is shallow, and the glands and vessels are few. Proœstrus is characterized by follicular growth in the ovary, while thickening of the mucosa, accompanied by congestion and multiplication of the stromal capillaries, takes place in the uterus. The glands of the mucosa begin to secrete. According to Marshall and Halnan, the

mammary tissue in the virgin bitch shows no growth characteristic of proœstrus or œstrus.

During œstrus, ovulation takes place and the growth phase in the uterus is superseded by a regressive one, which is characterized by the breakdown of the capillary walls and the extravasation of blood into the stroma. Aided by a certain amount of destruction of the superficial epithelium, the blood

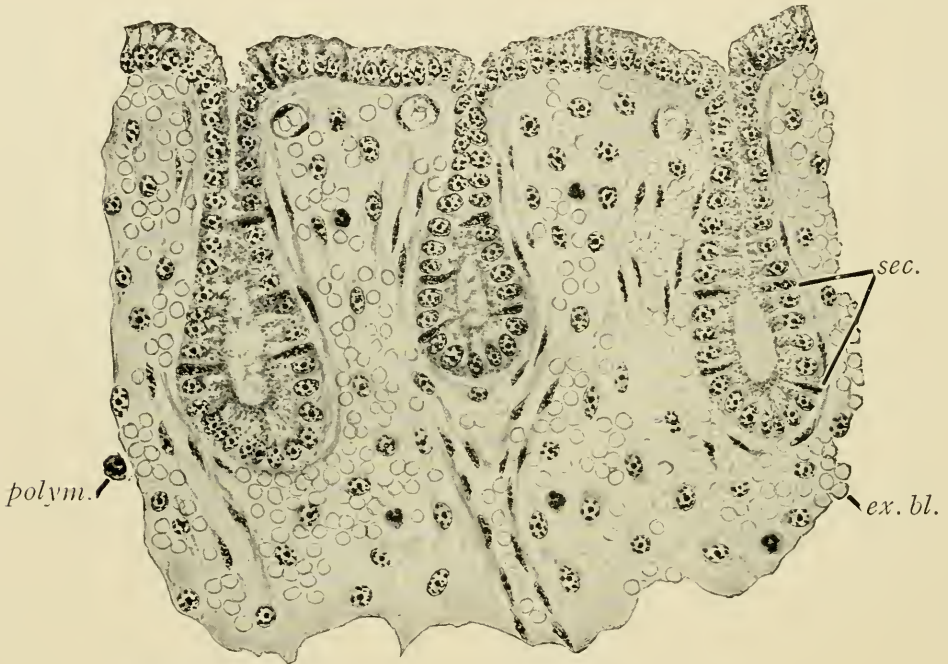


FIG. 15.—PROËSTROUS UTERINE MUCOSA OF DOG.

(From Marshall and Jolly).

*ex.bl.* extravasated blood corpuscles; *polym.* polymorph; *sec.* cells probably indicating secretory activity.

corpuscles find their way into the lumen of the uterus and thence to the exterior, giving rise to the external bleeding characteristic of early œstrus in the dog. During metœstrus the corpora lutea are beginning to form in the ovary, and the uterine mucosa regenerates. If pregnancy does not occur, pseudo-pregnant changes take place, correlated with the development and persistence of the corpus luteum in the ovary. Pseudo-pregnancy is characterized by considerable enlargement of the uterus and by growth of the mammary glands. Gerlinger distinguishes two layers of the uterine stroma during this period ;

a deep spongy foundation, and a compact superficial layer crowded with shallow crypts. At about eight to nine weeks after Œstrus, correlated with the atrophy of the corpus luteum, regressive changes take place in the accessory organs. These

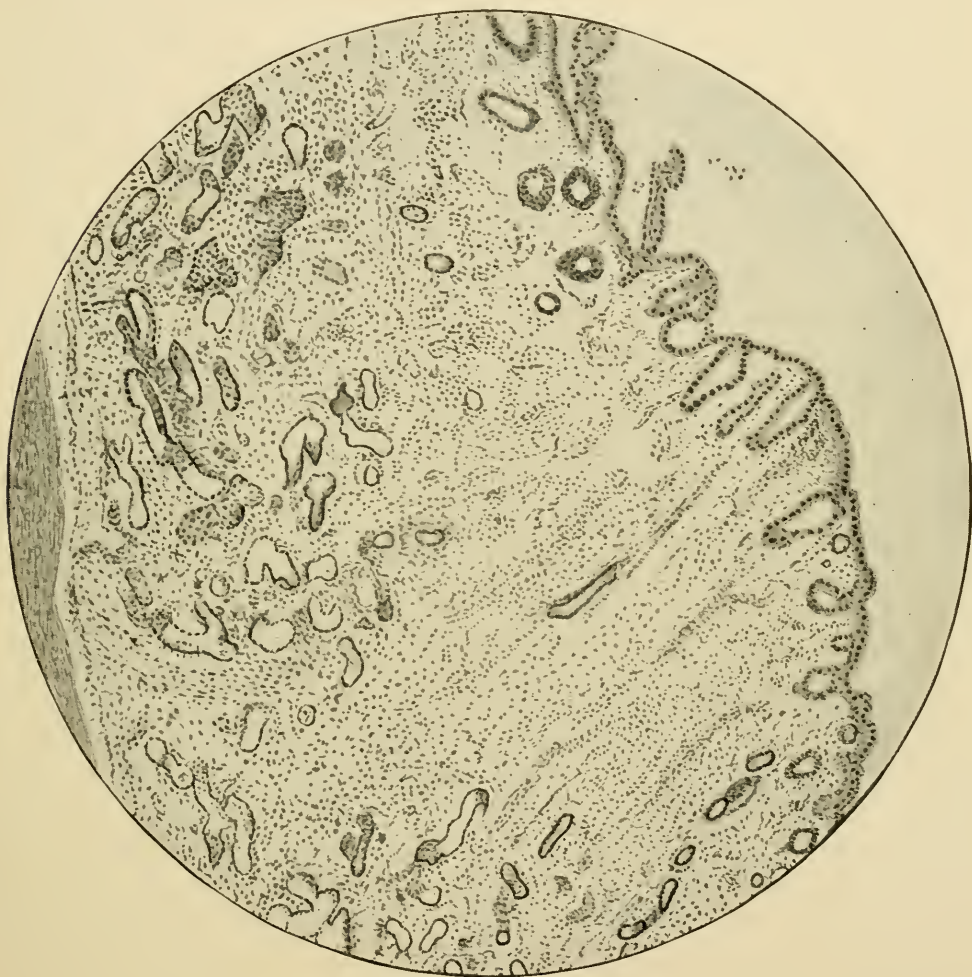


FIG. 16.—UTERINE MUCOSA OF DOG AT THE END OF PSEUDO-PREGNANCY.

Extravasated blood is seen in the stroma.

(From Marshall and Halnan).

changes lead to the secretion of milk in the mammary glands, and to the breakdown of capillaries and to the extravasation of blood into the stroma of the uterus. In spite of a similarity to the proœstrous phenomenon, this pseudo-pregnant degeneration in the uterus is readily distinguished by a difference in the



epithelium and glands. At the end of pseudo-pregnant degeneration, anæstrous quiescence supervenes.

In the early stages of pregnancy, changes similar to those of pseudo-pregnancy are found. Subsequently, of course, the development of both uterus and mammary glands is in excess of that found in the non-pregnant animal. Lactation occurs in what would be the next anæstrous period, so the complication of coincident œstrus and lactation does not appear. For the same reason the bitch, unlike rodents, cannot suckle and gestate at the same time.

### (c) GUINEA-PIG AND COW

The guinea-pig and cow both have a diœstrous cycle containing a luteal phase, which has been shown experimentally to be under the control of the corpus luteum. They are also similar in having a relatively long period of gestation, so that the young of both are born in an advanced stage of development.

*Guinea-pig.* Early workers on the œstrous cycle in the guinea-pig include Bischoff (67), Reichert (528), Hensen (289), Rein (532), and Rubaschkin (539). These workers all recognised that 'heat' occurred very soon after parturition and that ovulation was spontaneous. They failed, however, as did early workers on the rat and mouse, to trace the cycle without taking parturition as the starting-point. During the last twenty years very extensive investigations on the uterine, ovarian, and mammary cycles in the guinea-pig have been carried out by Loeb (379, 389, 391, 393), while more recently the whole subject has been reinvestigated by Stockard and Papanicolaou (599), who elucidated the vaginal cycle and introduced the vaginal smear technique. According to Stockard and Papanicolaou the length of the diœstrous cycle is about sixteen days (or rather longer in winter); Loeb's observations agree roughly with this figure, except that he found greater variability and a tendency towards long cycles after copulation. Voss (625) gives the length of the whole cycle as varying between thirteen and twenty days, of which diœstrus occupies nine to sixteen days.

Sterile copulation exerts no such influence on the time of appearance of the next œstrous period as it does in the rat

and mouse. This is probably correlated with the fact shown by Loeb that the corpora lutea function in postponing the next œstrus, even in the unmated cycle of the guinea-pig.

The guinea-pig is similar to the rat and mouse in having an immediate post-partum œstrous period, but it differs from these two animals, according to Loeb and Kuramitsu (406), in that inhibition of œstrus is not found during the rest of lactation. The second post-partum œstrus in the guinea-pig occurs in about sixteen days, *i.e.* at the normal interval. Lactation, in other words, fails to cause unusual persistence of the corpora lutea from the immediate post-partum ovulation. Since the mouse requires to be suckling more than two young for this result to be produced, the difference in the guinea-pig is probably accounted for by the relatively small demand made on the mother by the smaller-sized, more mature litter.

The ovarian cycle in the guinea-pig is rather remarkable, according to Loeb (398), who has described waves of follicular growth during both diœstrus and pregnancy. Atresia, however, not ovulation, results from this growth. Following ovulation, small follicles become medium-sized in some six days and large in another two, after which atresia sets in. The next wave of follicular growth culminates at the following œstrus and results in ovulation of some of the mature follicles and atresia of the remainder. During pregnancy there are two or more waves of follicular growth ending in atresia. The corpus luteum becomes fully formed about five days after ovulation. Regressive changes are first visible on the tenth day and accelerate rapidly until the next œstrus. By ten days after the new ovulation, the corpora lutea of the previous œstrus are reduced to small vacuolar bodies surrounded by a connective tissue capsule. During pregnancy the developmental stage of the corpora lutea lasts longer and results in the formation of larger structures. Degenerative changes, similar to those found in the diœstrous corpora lutea, are observed only after the fortieth day.

The post-ovulation condition of the uterus has been described at length by Loeb. According to his account, there is no marked hypertrophy such as takes place during the luteal phase in the dog, but definite changes occur in the epithelium and stromal glands of the uterus, and in its physiological condition.

The vaginal cycle of the guinea-pig is remarkable for the fact that during the diœstrous interval the vaginal orifice is normally closed by an epithelial membrane. This is ruptured at the approach of proœstrus by the turgidity of the vulva. Stockard and Papanicolaou have divided proœstrus and œstrus (which together last about twenty-four hours) into four stages according to the nature of the vaginal contents (Table 2).

TABLE 2.—VAGINAL CHANGES DURING ŒSTRUS  
IN THE GUINEA-PIG

(After Stockard and Papanicolaou).

Stage	Nature of contents of Vagina	Duration (hrs.)	Condition of Ovary	Condition of Vagina
I	Mucous secretion Epithelial cells (cornified)	6-12 hrs.	Mature follicles	Vaginal epithel- ium being shed. Infiltration of leu- cocytes beginning
II	Cheesy. Great num- bers of epithelial cells (nucleated)	2-4 hrs.	Ovulation	Leucocytes in- creasing. Desqua- mation of epithe- lium continuing.
III	Fluid thinner. Leu- cocytes beginning to appear	4-6 hrs.		Congestion. Leu- cocytes passing to lumen.
IV	Large numbers of leucocytes. Some hæmorrhage	1-2 hrs.	Corpus luteum forming	Rupture of few capillaries, fol- lowed by regenera- tion of epithelium.

Copulation, accompanied by the formation of the vaginal plug, takes place during the first stage, and the corpora lutea are fully formed four to five days later. Stockard and Papanicolaou consider the breakdown of the vaginal epithelium to be due to the removal of the protective influence of the corpora lutea of the previous ovulation, and to be homologous with human menstruation. The time relation of ovulation is, however, radically different from that found in the human.

Loeb and Hesselberg (402) have described a growth cycle in the mammary glands of the non-pregnant guinea-pig corresponding with the diœstrous cycle, a burst of growth accompanying each œstrus. Following œstrus, the glands regress and there is no reactivation during the luteal phase of the cycle.



Thus, during the time of maximum development of the corpora lutea in the non-pregnant animal, the mammary gland is at its minimum, so that the luteal phase of mammary growth is presumably missing in the normal non-pregnant guinea-pig. Prolongation of the life of the corpora lutea by hysterectomy or by deciduomata formation results in considerable mammary proliferation.

*Cow.* The importance of the cow from an agricultural point of view has attracted a large number of workers, among whom may be mentioned Schmaltz (547), Küpfer (336), Zeitzschmann (645), Murphey and co-workers (468-9), Frei and Metzger (232) and more especially Hammond (265), from whose work the following account is largely compiled.

The complete diœstrous cycle in the cow lasts about three weeks, of which œstrus (as determined by discharge of mucus and willingness to copulate) occupies twelve to twenty-four hours. Much variation is, however, found according to breed, age, condition of animal, and time of year. Ovulation is spontaneous, and occurs 0-24 hours after the beginning of heat. Only one ovum is normally produced at each ovulation. The ovaries generally ovulate alternately, though this is not invariable. The rupture of the follicle is accompanied by a small hæmorrhage. Three days after the beginning of heat the ovaries contain the young corpus luteum, an old corpus luteum from the last cycle (usually in the opposite ovary), and a number of small follicles. Eight days after 'heat,' the old corpus luteum has become insignificant, the new one is fully formed, and a new large follicle has appeared (usually in the same ovary as the old corpus luteum). Ten days later, *i.e.* three days before the beginning of the next heat, the ovaries are in much the same condition, except that the old corpus luteum has practically disappeared, and slight growth has occurred in the new large follicle. In another two days (one day before heat) degenerative changes, accompanied by decrease in size, have appeared in the recent corpus luteum, and rapid follicular growth is taking place.

The ovarian cycle in the cow is shown diagrammatically in Fig. 17, taken from Hammond. This diagram suggests that the follicle which will ovulate at the next œstrous period can already be distinguished from the 'reserve' of small follicles at the time

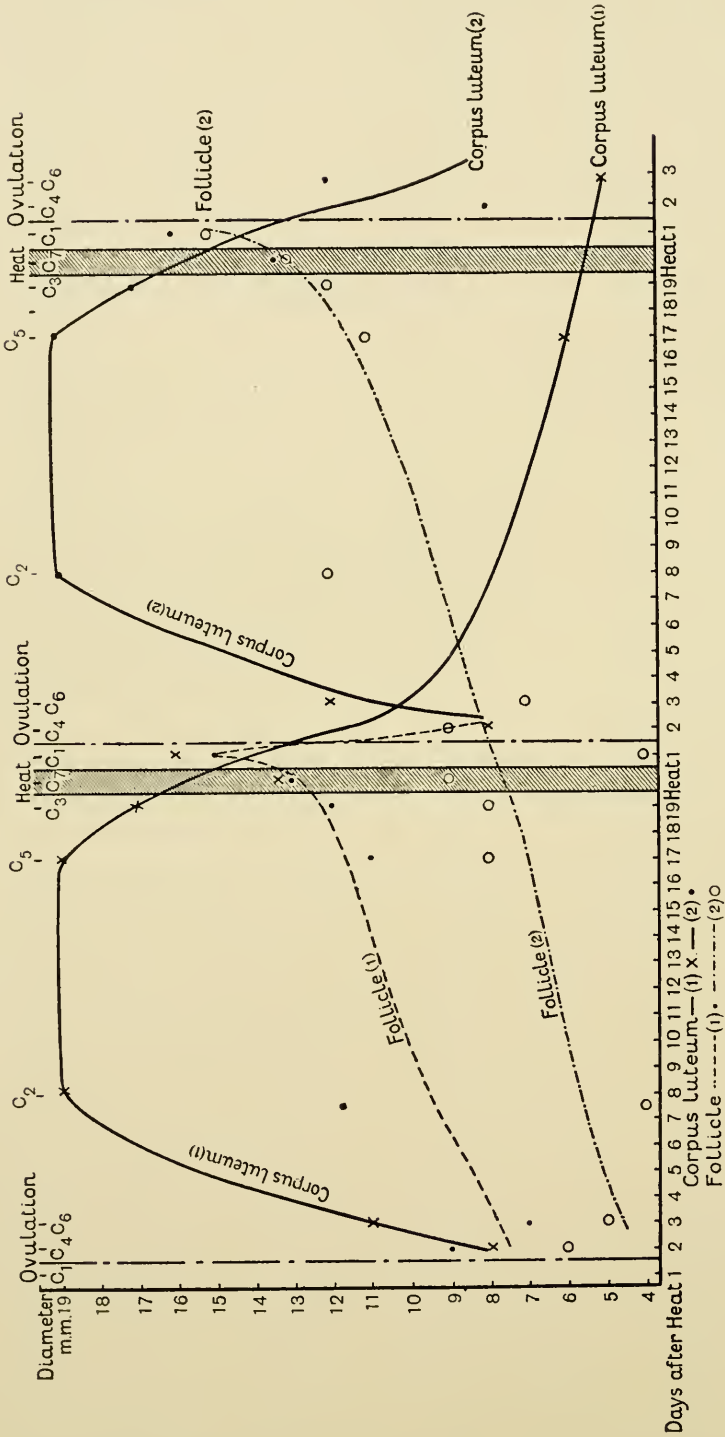


FIG. 17.—DIAGRAM OF OVARIAN CYCLE IN NON-PREGNANT COW.  
(From Hammond).

of the previous ovulation. Thus the cow differs from the mouse (see p. 137) where the follicles which will ovulate at the next Œstrous period become distinguishable only during the first half of the preceding diœstrus.

The cyclic changes in the accessory organs of the cow do not appear to have been entirely worked out. Hammond describes the uterus as secreting a large amount of fluid at œstrus (as occurs in other mammals) and showing congestion during the eight days following heat. According to Murphey the vaginal epithelium undergoes development up to eighteen days after the beginning of the previous heat, and this is followed by general desquamation and reformation of the epithelium during the next week. Hæmorrhage from the vaginal stroma, accompanied by leucocytic infiltration, is usual after heat. A more detailed description is given by Frei and Metzger, whose illustrations of the vaginal contents suggest that some proœstrous cornification may occur, as in the rat and mouse. The degree of cornification must be inconsiderable, however, compared with that found in the rodents.

Hammond has described cyclic changes in the mammary gland of the virgin animal correlated with the Œstrous cycle. Before puberty the glands consist merely of ducts, but after the first ovulation there occurs development of lobules of alveoli associated with, but lagging behind, the development of the corpus luteum. The gland never returns to the pre-pubertal state, but regression of the alveoli takes place during the second half of each diœstrus. Hammond does not describe any growth characteristic of the actual period of heat.

Lactation in the cow does not appear to have any effect upon the recurrence of the cycle in the ovary. Cows are normally put to the bull soon after parturition, and milking is continued until six to eight weeks before the next calf is due.

#### (d) HORSE, SHEEP AND PIG

These animals under conditions of domestication have a diœstrous cycle which probably contains a luteal phase controlled by the corpus luteum, but precise experimental information is lacking.

*Horse.* Until recently very little accurate information was available as to the œstrous cycle in the mare. Heape (287) made the general observation that the length of the diœstrous cycle is three to four weeks, while Marshall (444) states that the duration of œstrus is about a week, becoming shorter, according to Ewart (189), as the season advances.

Lately, Seaborn and Champy (553), Seaborn (552), and Aitken (4) have brought forward much more data.

Seaborn gives the length of the diœstrous cycle as twenty-four days, while Aitken found the average to be twenty-two or twenty-three days, with a normal variation from twenty to twenty-five days. The duration of proœstrus and œstrus is three days each, according to the former authors. Copulation takes place only during œstrus. Aitken gives the duration of œstrus as seven days, with normal variation from four to eleven days. Both agree that ovulation occurs towards the end of œstrus.

The ovary of the horse is rather remarkable for its size and fibrous nature. Ovulation, which is spontaneous at œstrus, takes place from an ovulation fossa. According to Aitken, germinal epithelium covers this part of the ovary, while the rest is covered by peritoneum. Aitken gives the diameter of the mature follicle as about 6 cms. and of the fully developed corpus luteum as 4 cms. An ovary containing a ripe follicle weighs about 300 gms. and one during diœstrus about 60 gms. Ovulation is not necessarily alternate, and Aitken found a high proportion of double ovulations, often from one ovary. The maturation of the follicle, and its differentiation from the group of small follicles, seems to occur very rapidly before ovulation. Regressive changes take place in the corpus luteum during the second half of diœstrus.

The uterine cycle has been roughly described by Seaborn (552), the central point being the usual proliferation of the mucosa at proœstrus. No vaginal cycle has so far been described and Aitken found that vaginal smears showed no definite cycle. As Marshall states that the mammary gland becomes congested and enlarged during œstrus, it would appear that a cycle exists in this organ in the non-pregnant animal.

*Sheep.* The sheep is a good example of the transition from the monœstrous to the polyœstrous condition. Most wild



species, according to Marshall (444), are probably monœstrus, having only one œstrous period in the short breeding season. In captivity, however, and particularly under conditions of domestication, two or more diœstrous cycles appear during the breeding season, while the Australian Merino is said to experience an unbroken series of diœstrous cycles throughout the year in the absence of pregnancy. In Great Britain the breeding season lasts from two to three months. According to Marshall the length of the cycle is about fifteen or sixteen days, though this doubtless varies according to breed and nutrition. Other authors have given from two to four weeks as the length of the cycle. Neither vaginal nor mammary cycles have been described in the sheep, but Marshall (440) has given a full description of the changes taking place in the non-pregnant uterus. These changes consist essentially of the phases of rest, growth, destruction and regeneration, such as have been described in the dog.

During the growth phase the mucosa increases in thickness, the stroma undergoes cell division, and uterine congestion begins. The period of destruction is characterized by the breaking down of some of the capillaries and extravasation of blood into the stroma. Bleeding into the uterine cavity does not usually occur. The extravasated blood usually remains under the endometrium, giving rise to pigmentation. This period of destruction, corresponding to the end of proœstrus, is followed by a period of recuperation. Subsequently the uterus returns to the condition of rest.

*Pig.* The duration of the diœstrous cycle in the sow varies from two to four weeks, but is usually three weeks. Struve (604), in a statistical investigation, found the average to be  $20.66 \pm .205$  days. The external signs of heat are excitement of the animals, and swelling of the vulva, from which there may be a slight (possibly sanguinary) discharge. According to Corner (122), however, the blood is of vulval rather than internal origin. Œstrus lasts about three days.

The ovarian cycle in the pig has been described in detail by Corner (122). About three days before the onset of œstrus, the follicles due to ovulate undergo rapid growth and finally attain a diameter of 8-10 mms. Ovulation occurs towards the end of œstrus according to Lewis (362), but Corner and Amsbaugh

(128) think that it is probably on the second day. The ova reach the uterus in about four days, and, if fertilization has taken place, become implanted eight to ten days after ovulation. The corpus luteum attains its maximum size in the non-pregnant animal in about ten days, at which time it is 8-9 mms. in diameter. The corpus luteum of pregnancy may enlarge to a diameter of 10-11 mms.

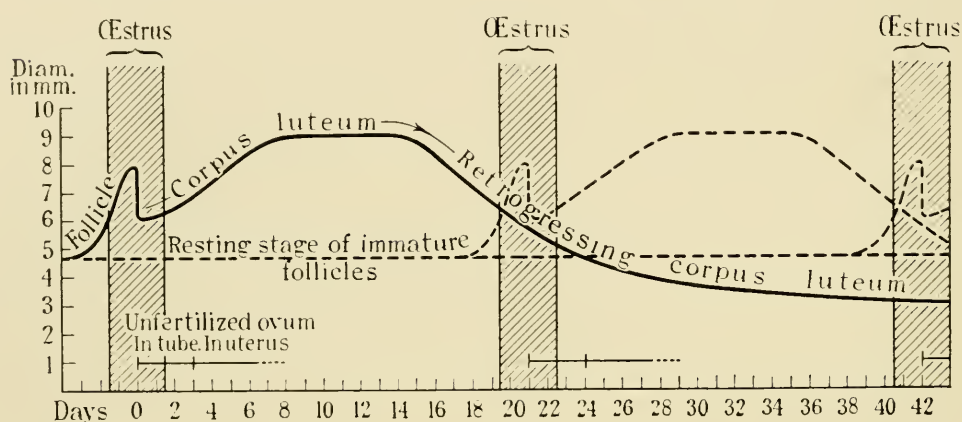


FIG. 18.—DIAGRAM OF OVARIAN CYCLE IN NON-PREGNANT SOW.  
(From Corner).

At about the tenth day after ovulation in the non-pregnant animal a comparatively sudden regression takes place in the corpus luteum, which in two or three days shrinks to less than half its previous volume, largely owing to the disappearance of the granulosa lutein cells. This degeneration is followed by the growth of the next group of follicles. The ovarian cycle in the unmated animal is shown by Corner as in Fig. 18.

The changes in the uterine mucosa corresponding to the ovarian cycle have been studied in detail by Corner. During œstrus the cells are of low columnar type and are closely packed together. Mitosis is frequent, and the mucosa has the appearance of active proliferation. The glands, however, do not show this. The sub-epithelial connective tissue is crowded with polymorph leucocytes. The uterine stroma is œdematous.

During the first week after œstrous growth, a further proliferation takes place in the mucosa cells, which, by the end of the first week, have become columnar. There is also a burst of mitosis in the gland cells, beginning three to four days after

ovulation. All mitosis, however, ceases about the end of the first week. At about the tenth day the mucosa cells are again reduced to the low columnar type, with the extrusion of cytoplasmic processes, and the gland epithelium returns to normal. During the last few days of diœstrus the mucosa cells become more elongated and large numbers of leucocytes appear in the stroma.

This building up of the uterine mucosa during the first half of diœstrus, though comparatively slight, is clearly correlated with the development of the corpus luteum, and is unaffected by the fate of the ova. It may be considered, in the non-pregnant animal, as an abbreviated pseudo-pregnancy.

Vaginal and mammary changes during the diœstrous cycle in the pig do not seem to have been described.

As in the cow, mare, and guinea-pig, lactation has no effect on the recurrence of the cycle, except that, according to Marshall (444), there is an interval of five weeks after parturition before the next œstrus. Struve (604), however, states that œstrus recurs four to nine days after parturition.

#### (e) MOUSE AND RAT

In spite of the ease with which the animals can be kept for observation, accurate data on the œstrous cycle of the rat and mouse have only recently become available. Earlier workers were much handicapped by the difficulty of external diagnosis of œstrus, and were compelled to use the immediate post-partum œstrus as their starting-point. Sobotta (583), working from this period, put the length of the cycle in the mouse at about twenty days, while Lataste (358), from the intervals between sterile copulations, placed the length of the cycle at about twelve days. Long and Smith (427), and Smith (572) estimated the length to be sixteen to nineteen days. In the white rat Morau (463), Lataste (358), Long and Quisno (426), and Heape (287), all put the length of cycle at ten days. The discovery, however, that a very definite vaginal cycle exists in both these animals has made it possible to analyse completely the length of the œstrous cycle. In the ordinary unmated mouse and rat œstrus occurs about every five days. Long and Evans (425) for the rat give an average length

of cycle of 5.4 days for 1,999 cycles. Of these 82 per cent. had an average of 4.6 days. In the mouse the length of the cycle is given by Allen (6) as between four and five days, while the present author (498) found a mean length of 6.2 days for 1,000 cycles. The first œstrus occurs in the rat when about ten to eleven weeks old, while in the mouse the cycle begins at the age of about seven weeks. The earlier cycles appear to be slightly longer than those occurring in the fully mature animal. In the mated mouse, in the absence of pregnancy, the interval between sterile copulations is about twelve days, and this increase is due to the occurrence of a pseudo-pregnant period. Long and Evans found the interval in the rat to be about thirteen days and also showed that the mechanical stimulation of the cervix by the vaginal plug results in the ovarian changes associated with the pseudo-pregnant period (see also p. 171). Since copulation was originally used as the criterion of œstrus, this lengthening of the cycle after sterile copulation explains the error in the original estimates.

œstrus occurs within twenty-four hours after parturition and is then in abeyance during the whole of lactation, provided that a normal-sized litter is being suckled. In the mouse this diœstrous period during lactation lasts for about three weeks (491). In the rat, it lasts rather longer, twenty-five to forty days according to Long and Evans, varying with the number of young suckled. This disappearance of œstrus during lactation is correlated with ovarian changes which result from lactation. The abnormal prolongation of lactation does not result in further prolongation of the diœstrous period. By foster-mothering young litters, it is possible to prolong lactation in the mouse for forty to fifty days without materially extending the length of the lactation diœstrus (495). If one or two young only are being suckled, inhibition is not set up by lactation and the cycle after parturition is normal. If pregnancy supervenes from the immediate post-partum œstrus the female mouse and rat may be both pregnant and lactating at the same time. Under these conditions the length of pregnancy in the mouse is increased from the normal nineteen or twenty days up to as much as twenty-eight days. According to Kirkham (323) this is due to delay in implantation of the embryos.



The period of œstrus is marked by very definite changes in the vagina and uterus, and by follicular maturation in the ovaries. Changes in the mammary gland may also occur during the ordinary unmated cycle.

*Ovarian cycle.* The morphological picture of the ovarian cycle is complicated by the fact that the corpora lutea survive histologically long after their function is in abeyance, and in the non-mated adult animal four or five sets of corpora lutea,

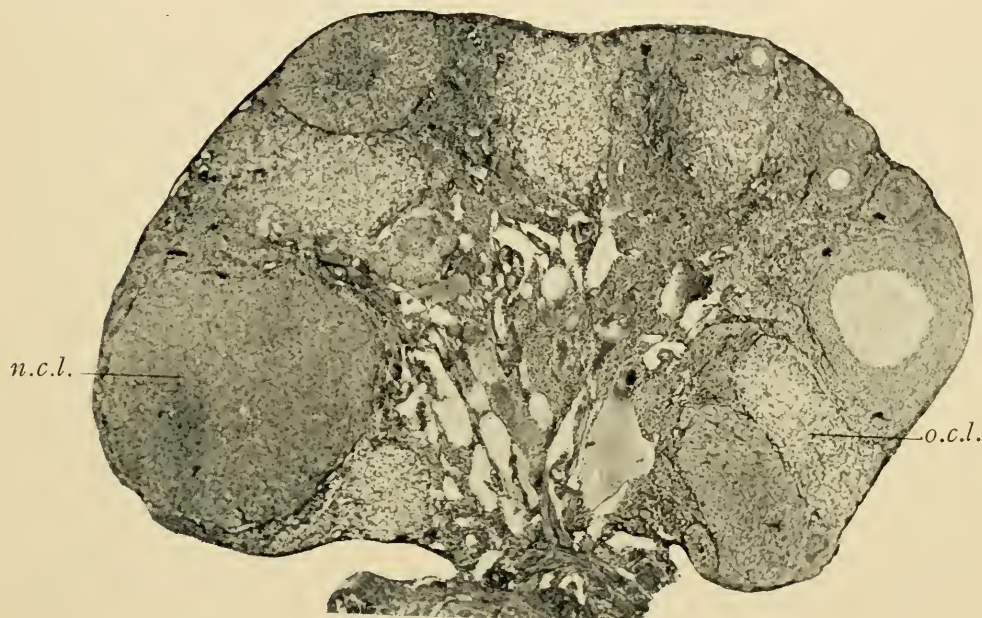


FIG. 19.—OVARY OF MOUSE (PASINI STAIN), SHOWING TWO SETS OF CORPORA LUTEA.

*n.c.l.* new corpora lutea; *o.c.l.* old corpora lutea.

representing previous ovulations, may be found at one time. A large number of medium-sized follicles are present in the ovary of the mouse at the beginning of diœstrus, and in this large group it is difficult or impossible to pick out the set destined to ovulate at the next œstrous period. At about the middle of diœstrus, however, certain of the follicles undergo a rapid phase of growth. This growth phase becomes more rapid during proœstrus, and culminates in ovulation during the period of œstrus—late in œstrus according to Allen (6), but earlier according to other workers (82). Long and Evans state that in the rat ovulation may occur any time after eighteen hours from

the beginning of vaginal cornification. The passage of the ova down the tube is facilitated in both animals by the accumulation of fluid in the periovarian cavity at œstrus. During the next two days the corpora lutea develop, and in the unmated normal rat they attain their maximum growth about three days after œstrus. This set of corpora lutea may be distinguished histologically from the previous sets without the aid of vital staining, but the previous sets can only be separated reliably by means of this technique, or by special histological examination of the

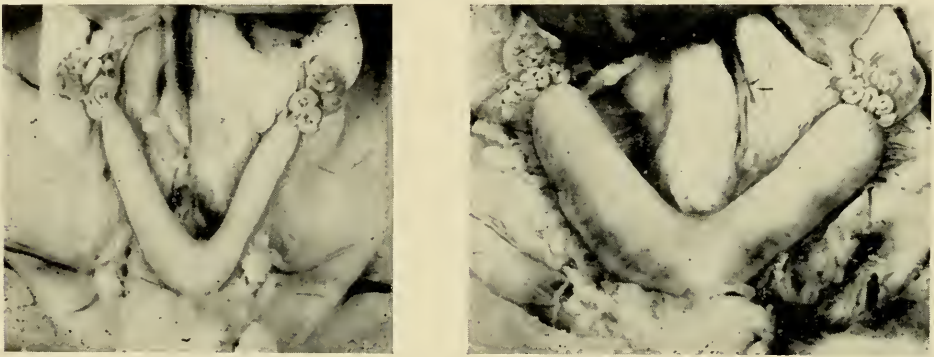


FIG. 20.—UTERUS OF MOUSE: (a) DURING DIÆSTRUS, (b) DURING (ÆSTRUS, SHOWING TYPICAL DISTENSION OF UTERUS AND OVARIAN CAPSULE DURING (ÆSTRUS.

lutein granules. According to Long and Evans, degeneration can be detected by the latter technique in the new corpora lutea at the beginning of the next œstrus. During pseudo-pregnancy growth of the follicles is postponed until about the usual time before the next œstrus. This postponement of growth is undoubtedly brought about by the activity of the corpora lutea, which undergo greater development during pseudo-pregnancy than in unmated animals. In the same way ovulation is entirely inhibited during true pregnancy, and follicular maturation, beginning when the corpora lutea atrophy just before parturition, is completed only in time for the post-partum ovulation. No ovulation takes place between the œstrous period occurring immediately after parturition and that marking the end of the lactation diœstrus.

*Uterine cycle.* The uterine cycle in both the mouse and rat is closely synchronized with the ovarian cycle. During diœstrus



the uterus is constricted and anæmic. At proœstrus dilation of the lumen has begun and at the time of ovulation the uterus has increased to about twice the normal diameter; the stroma and muscular material are then very much attenuated. The increase in size is caused solely by the distension of the lumen.



FIG. 21.—UTERUS OF MOUSE DURING DIOESTRUS.

During metœstrus the uterus gradually returns to its diœstrous size, and in the unmated animal no further change takes place until the next proœstrus. During pseudo-pregnancy, however, growth of the stroma takes place, and at the end of pseudo-pregnancy, correlated with the atrophy of the corpora lutea, a certain amount of hæmorrhage into the lumen may occur.

*Vaginal cycle.* The vaginal changes during the œstrous cycle are also very pronounced and are now used for the detection of the œstrous cycle in the intact animal. During proœstrus the

vaginal mucosa undergoes growth and becomes many layers thick. The subsequent degeneration of this growth takes the form of keratinization of the cells, which are subsequently sloughed off into the lumen and can be collected for examination. This stage is followed by the infiltration of vast numbers of

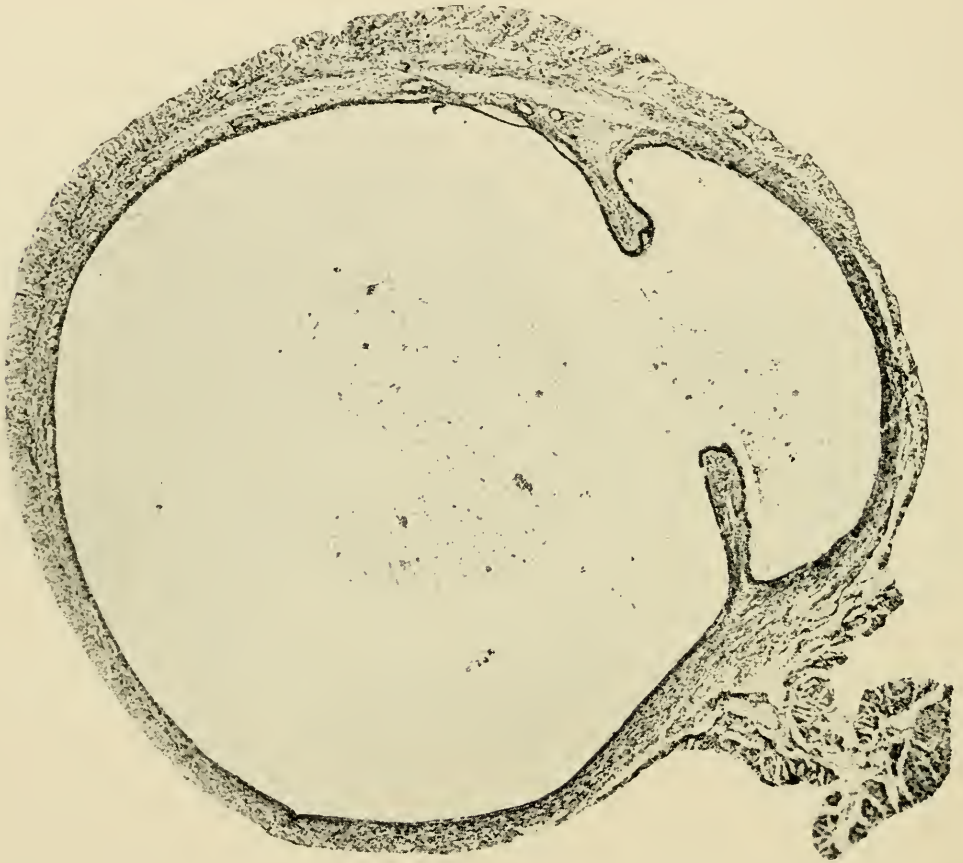


FIG. 22.—UTERUS OF MOUSE DURING ŒSTRUS, SHOWING DISTENSION OF LUMEN.

leucocytes from the vaginal stroma. The vaginal contents, therefore, show typical cyclic changes, which for the mouse may be summarized as follows :

*Diastrus.* Epithelial cells, largely nucleated, and leucocytes constitute most of the vaginal smear during this stage. The smear tends to be fairly fluid in the unmated animal.

*Proœstrus.* During this stage the leucocytes disappear altogether from the vaginal contents and the smear is made up entirely of lightly staining nucleated epithelial cells. The



vaginal contents are serous during this stage, which lasts some twelve hours in the normal mouse.

*Œstrus.* Towards the end of proœstrus the nucleated epithelial cells begin to be mixed with the cornified mucosa cells and the latter gradually supersede the former in the vaginal contents. The cornified cells are typically non-nucleated and eosinophil at this stage, while the vaginal contents as a whole have a granular appearance. Copulation occurs at this stage,

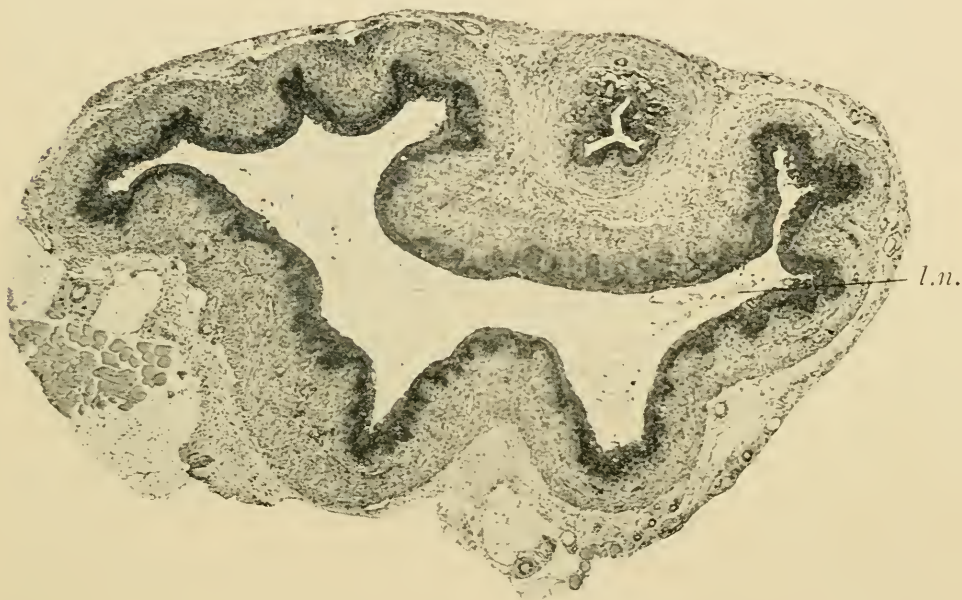


FIG. 23.—VAGINA OF MOUSE DURING DIŒSTRUS.

*l.n.* leucocytes and nucleated epithelial cells in the lumen.

usually within the first day. As in the rat, the formation of the vaginal plug from the vesicular secretion of the male provides a reliable means of detecting copulation. The plug, surrounded by an envelope of cornified vaginal epithelium, is shed in about eighteen to twenty-four hours. In the unmated animal this stage lasts about two days and is terminated by the appearance of leucocytes in the smear.

*Metœstrus.* During metœstrus the vagina returns to the diœstrous condition. The smear consists of cornified cells with a gradually increasing number of leucocytes. Finally, nucleated epithelium appears, and the smear becomes of the normal diœstrous type.

*Pregnancy.* During the whole of pregnancy the smear consists of a few nucleated epithelial cells and leucocytes. Much mucus is present all the time, and blood appears in both the rat and mouse at about the twelfth day owing to uterine hæmorrhage (the 'placental sign' of Long and Evans).

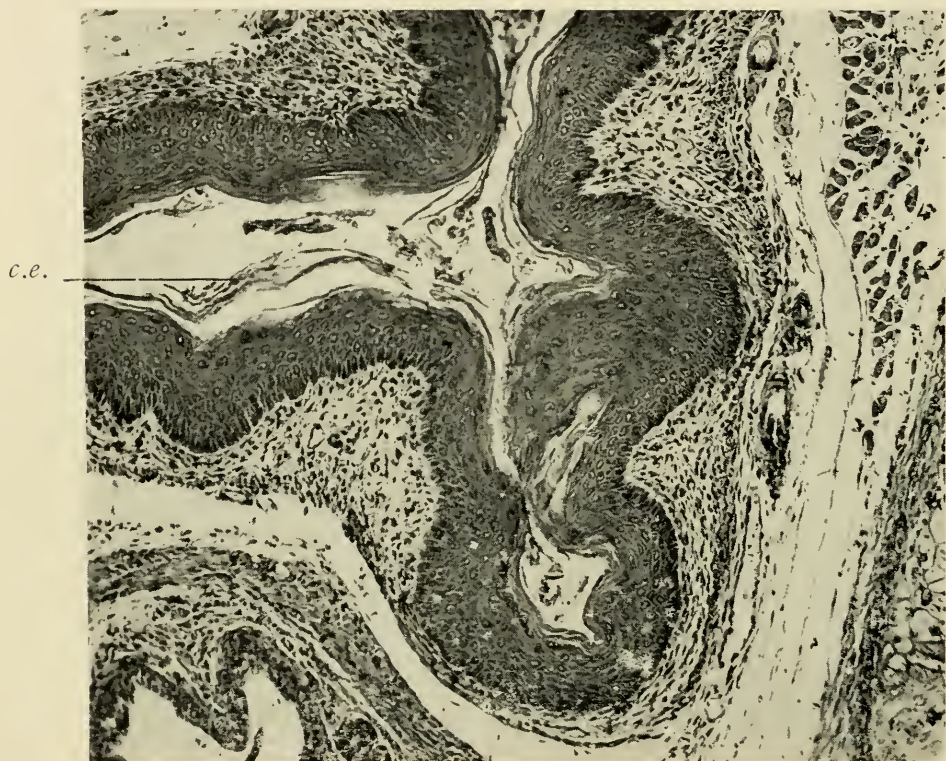


FIG. 24.—VAGINA OF MOUSE DURING ŒSTRUS, SHOWING CORNIFICATION OF THE EPITHELIUM.

*c.e.* cornified epithelium sloughed off into the lumen.

*Pseudo-pregnancy.* Following sterile copulation, *i.e.* during pseudo-pregnancy, the vaginal smear is of the dioestrous type, except that much mucus is present. About three days before the next œstrus is due a smear containing blood is usually obtained.

In the rat the vaginal cycle is similar to that of the mouse. Long and Evans (425) summarize the changes during the cycle in the rat as in Table 3.

The nature of the leucocytes in the vaginal smear has been studied by Post (518) and Guttmacher (254).



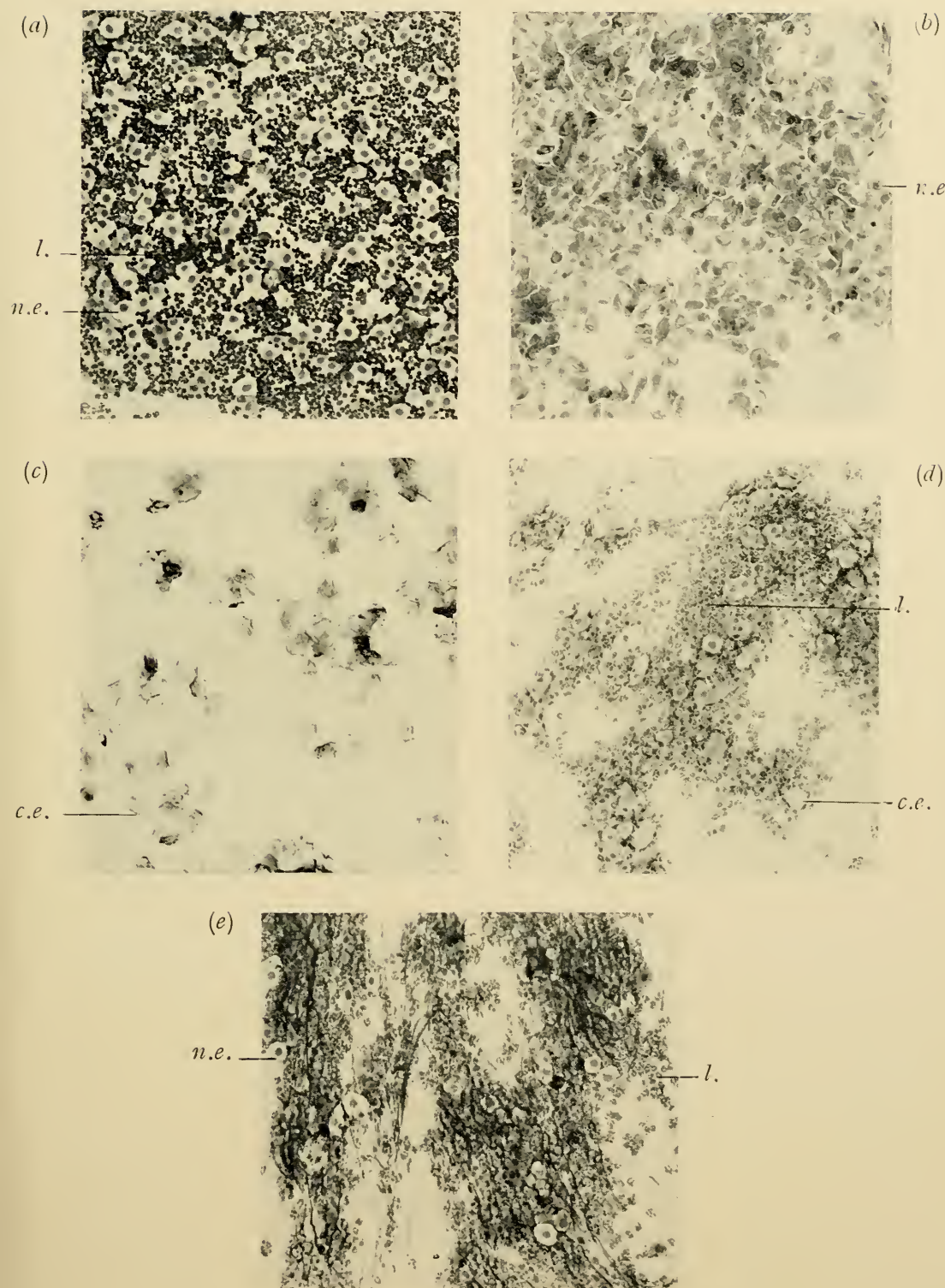


FIG. 25.—VAGINAL SMEARS OF THE MOUSE DURING THE ŒSTROUS CYCLE.  
 (a) diœstrus, (b) late proœstrus, (c) œstrus, (d) late metœstrus, (e) pregnancy.  
 c.e. cornified epithelial cells; l. leucocytes; n.e. nucleated epithelial cells.

TABLE 3.—SUMMARY OF ŒSTROUS CYCLE CHANGES IN THE RAT.  
(From Long and Evans).

Stage	Living Animal	Histology of Vaginal Mucosa	Uterus	Ovary and Oviduct
1 (12 hrs.)	Vaginal mucosa slightly dry. Smear of epithelial cells only. Lips a little swollen. In heat towards end.	Many layered (8-12) .08-1 mm. Mitoses active. Cornified layer under superficial layer of epithelium. No leucocytes.	During Stage 1 uterus becomes distended with fluid increasing in diameter from 2.3-3.7 mm.	Follicles large.
2	Vaginal mucosa dry and lustreless. Smear of cornified cells only. Lips swollen. In heat.	7-11 layers of cells. .08-.1 mm. thick. Cornified layer well formed and superficial. No leucocytes. Mitoses fewer.	Reaches greatest distention (5 mm.) and thinness of epithelium and then retrogresses to diameter of 1.8 mm. Vacuolar degeneration sometimes begins.	Follicles largest. Eggs may undergo maturation.
3 (2 and 3 27 hrs.)	As in Stage 2, but cornified material abundant (cheesy) and animal not in heat.	5-9 cells thick. .064 mm. thick. Cornified layer loose and finally completely detached. No leucocytes. Mitoses still fewer.	Diameter of uterus about 2.0 mm. Epithelium undergoing vacuolar degeneration.	Ovulation. Secretion of fluid into periovarial space and oviduct.
4 (6 hrs.)	Vaginal mucosa slightly moist. Smear of cornified cells and leucocytes. Swelling of lips gone.	4-8 cells thick. .062 mm. thick. Cornified layer gone. Many leucocytes. Mitoses increasing.	Diameter of uterus 2.2 mm. Some vacuolar degeneration, but also regeneration.	Young corpora lutea. Eggs in oviduct. Follicle smallest.
5 Diœstrous interval (57 hrs.)	Vaginal mucosa moist, glistening. Smear of leucocytes and epithelial cells. Variable amount of mucus.	4-7 cells thick. .042 mm. thick. Leucocytes. Mitoses not numerous.	Diameter 1.7 mm. Epithelium undergoing regeneration.	Follicles of various sizes. Corpora lutea continue to grow. Eggs traversing oviduct throughout early interval.



*Mammary Cycle.* The mammary cycle in the unmated rat and also the development of the gland during pregnancy have been dealt with by Myers (472) and Sutter (605). The former states: 'If pregnancy does not occur with the first ovulation the mammary glands undergo slight retrogressive changes; however, as the next ovulation approaches the mammary gland takes on a new development. Similar changes can be observed with each succeeding ovulation until pregnancy occurs.'

(f) RABBIT AND FERRET

The rabbit and ferret correspond in two peculiarities; œstrus persists throughout the breeding season in the absence of

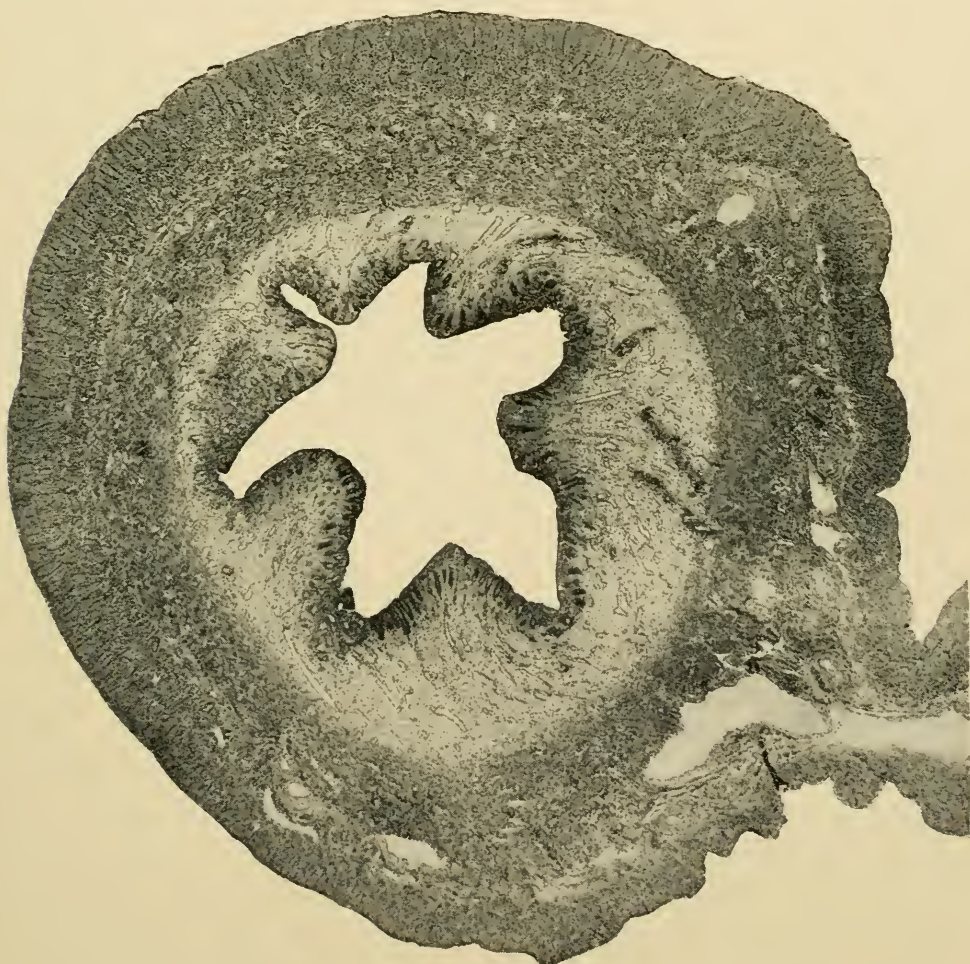


FIG. 26.—UTERUS OF RABBIT IN ŒSTRUS.  
The stroma is glandular, but compact.

copulation, and, as originally shown by Heape (288) in the rabbit, and Marshall (441) in the ferret, ovulation is dependent upon copulation. Both animals have a well-developed pseudo-pregnant period after sterile copulation, during which the ovary, uterus, and mammary glands undergo growth similar to that found during at least the first half of pregnancy.



FIG. 27.—UTERUS OF PSEUDO-PREGNANT RABBIT.

The stromal glands are greatly developed.

*Rabbit.* The histological and physiological changes occurring during the fertile cycle of the rabbit have been extensively studied by Ancel and Bouin (27, 29, 30-4, 75-7) and by Hammond (264). The changes which take place are very marked in the uterus, ovaries, and mammary glands. The vaginal cycle is less clear, though changes have been described by Tsu (617) and Courrier (139). Since œstrus is persistent, the only cycle is that of anœstrus, œstrus, pregnancy, and lactation. If the animal becomes pregnant at the beginning of the breeding season a condition of œstrus will reappear immediately after parturition and will continue during lactation. Pregnancy, however, does not usually occur during lactation, as the blastocysts fail to



become embedded and are reabsorbed. This sterility set up by lactation in the rabbit is clearly a continuation of the conditions found in the rat and mouse, where lactation causes a delay in the implantation of the blastocysts, but not a permanent inhibition. Sterile copulation in the rabbit is followed by a condition of pseudo-pregnancy, during which the changes in the ovary,



FIG. 28.—PHOTOGRAPH OF MAMMARY GLAND OF  
PRE-PUBERTAL RABBIT.

The gland is limited to a few ducts around the nipple.  
(After Hammond).

the uterus, and the mammary glands are similar to those occurring during the first half of pregnancy. Pseudo-pregnancy in the rabbit, though not so prolonged as in *Dasyurus* or the ferret, is nevertheless a far more distinct phase than the pseudo-pregnant period in the mouse and the rat. The female rabbit is atypical among lower mammals in allowing copulation at other times than œstrus (264).

The ovarian cycle consists in the maturation of Graafian follicles at the beginning of the breeding season and persistence of mature follicles until copulation takes place, or until the breeding season ends. In the absence of mating, atresia sets in at the end of the breeding season. Ovulation takes place about

ten hours after copulation. The subsequent development of the corpora lutea is continued for some fourteen days in the non-pregnant animal.



FIG. 29.—PHOTOGRAPH OF MAMMARY GLAND OF RABBIT IN FIRST ŒSTRUS.

The ducts have developed out radially from the nipple.

The uterine cycle consists of growth and vascularization at the beginning of the breeding season, and, following ovulation, in certain pregnancy or psuedo-pregnancy changes. During œstrus the uterine stroma, though hyperæmic, is not highly glandular and the epithelium is straight and continuous. After ovulation, under the influence of the corpus luteum, immense growth takes place in the glands, which supply a secretion responsible for the initial nutrition of the embryos. During



lactation the uterus becomes much atrophied, presumably owing to the drain on the metabolism. This atrophy during lactation is in marked contrast to the conditions found in the rat and mouse, in which the uterus is active during lactation owing to the persistence of the corpora lutea.



FIG. 30.—PHOTOGRAPH OF MAMMARY GLAND OF RABBIT  
TWELVE DAYS PSEUDO-PREGNANT.

Clumps of alveoli have developed along the ducts.  
(After Hammond.)

The cyclic changes in the mammary glands of the rabbit are also very obvious. Prior to the first Œstrous period the gland consists merely of a few ducts in the neighbourhood of the nipple. At the time of the first Œstrous period these ducts grow out radially for a distance of an inch or an inch and a half round

the nipple. At this stage, however, the development of the gland stops until ovulation has taken place and luteal activity begins. Under the influence of the corpus luteum the third phase of mammary development, the appearance of bud-like



FIG. 31.—PHOTOGRAPH OF MAMMARY GLAND OF RABBIT AFTER PSEUDO-PREGNANCY.

The lobules of alveoli are undergoing atrophy, but complete return to the virgin condition (Fig. 29) is not found.

structures at the end of the ducts and the growth of nests of alveoli, is started. In the absence of pregnancy no further development than this occurs, and the degenerative phase, which coincides with the atrophy of the corpora lutea at the end of pseudo-pregnancy, results in the appearance of a small



amount of milk in the ducts. During pregnancy the growth of the mammary glands for the first fourteen days is similar to that found during pseudo-pregnancy and is lateral and radial only.



FIG. 32.—PHOTOGRAPH OF MAMMARY GLAND OF RABBIT  
TWENTY-THREE DAYS PREGNANT.

Great hypertrophy of the gland, characterized by secondary thickening, has begun.

In the second half of pregnancy an entirely new phase of mammary growth is initiated, *i.e.* a thickening of the gland, during which the different nipple areas become confluent. The tissue reaches a thickness of some  $\cdot 3\text{--}\cdot 4$  cms., and the glands weigh about 100 gms. at the end of pregnancy.

*Ferret.* Reproduction in the ferret has been studied by Marshall (441) and Hammond and Marshall (267). The breeding season in the ferret lasts from about March to August, and ovulation can take place at any time during this period, provided that copulation occurs. Ripe follicles are always present in the unmated female during the breeding season.

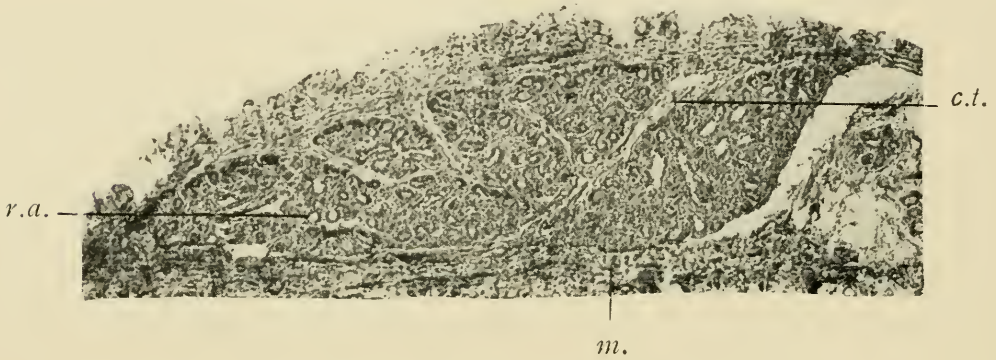


FIG. 33.—SECTION OF LOBULE OF MAMMARY GLAND OF RABBIT DURING PSEUDO-PREGNANCY.

*c.t.* connective tissue; *m.* muscle; *r.a.* rudimentary alveoli.

The pseudo-pregnant period which follows sterile copulation is remarkable in that it lasts six weeks, as long as true pregnancy. In this characteristic the ferret is without parallel among known Eutheria, except possibly the dog. Œstrus returns about eight weeks after sterile copulation.

The ferret is also peculiar in showing a prodigious growth of the vulva at œstrus. This swelling, which begins at the onset of œstrus, reaches its maximum size in two to three weeks, and is maintained during the whole of the breeding season in the absence of copulation. The hypertrophy, which enlarges the œstrous vulva to about fifty times the anœstrous size, disappears at the beginning of pregnancy, pseudo-pregnancy, or anœstrus.

The uterus also shows well marked development during œstrus, but this is continued and increased after ovulation. During anœstrus the uterus is very small and its glands comparatively undeveloped. At the beginning of œstrus the uterus enlarges, and the mucosa and glands are rather better developed. The uterus remains static in this condition during the whole



œstrous period. In pseudo-pregnancy, development continues up to the fifth or sixth week, after which degeneration takes place.

In virgin ferrets, the mammary glands are entirely undeveloped both in anæstrus and œstrus. During pseudo-pregnancy

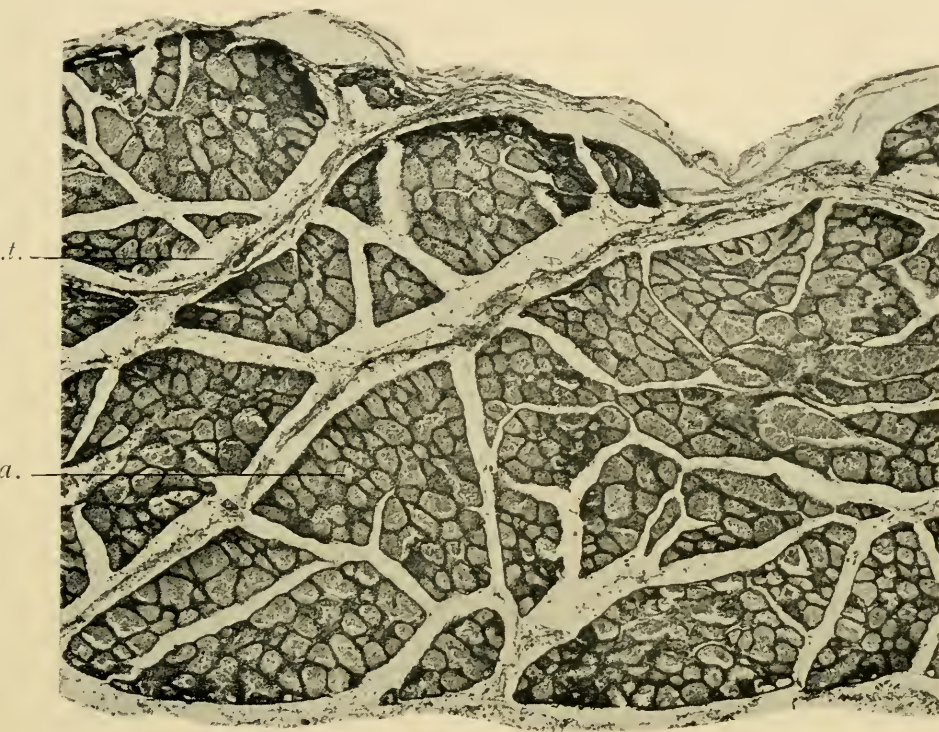


FIG. 34.—SECTION OF MAMMARY GLAND OF TWENTY-NINE DAYS PREGNANT RABBIT.

*c.t.* connective tissue; *l.a.* lobules of alveoli.

the ducts grow out round the nipple, and develop bud-like terminations consisting of secretory alveoli.

#### (g) PRIMATES

The most obvious stage of the œstrous cycle in Primates is, of course, the menstrual period, and most of the early work on the Primate cycle was directed to analysing the significance of this phenomenon. The correlation of the ovarian and uterine cycles has been attempted systematically only during recent years. Little success has been attained, and Heape's original observa-

tion on *Semnopithecus entellus*, that a regular menstrual cycle may be maintained in the apparent absence of an ovarian cycle, has been fully substantiated.

*The uterine cycle.* The uterine cycle in at least two species of monkey and in the human has been fully worked out. The writings of Heape on *Semnopithecus* (284) and *Macacus rhesus*

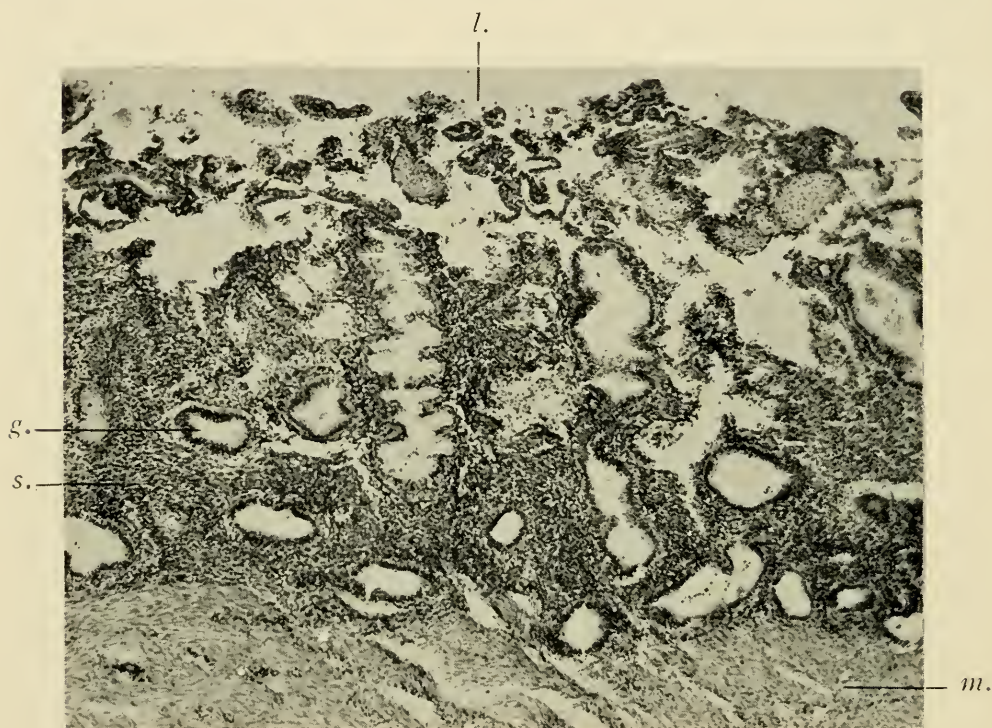


FIG. 35.—ENDOMETRIUM OF HUMAN UTERUS ON THE FIRST DAY OF MENSTRUATION, SHOWING DESTRUCTION OF EPITHELIUM AND SUPERFICIAL STROMA.

*g.* gland; *l.* lumen; *m.* muscle; *s.* stroma.

(After Shaw).

(285), of Corner (123) and Allen (12, 14, 15, 16) on *Macacus*, of Van Herwerden (297) on *Cercocbus*,<sup>1</sup> and of Hirschmann and Adler (307), Webster (632), Corner (125, 126), and Shaw (259) on the human should be consulted for details and references.

The uterine cycles in monkeys and women appear to be identical in all essential features, and Milnes Marshall's (456) original division of the phases in the human, identical with

<sup>1</sup> More probably *Macacus cynomologos*.



Heape's division in *Semnopithecus* and *Macacus*, distinguished four main stages :

- (a) period of rest.
- (b) period of growth.
- (c) period of destruction.
- (d) period of regeneration.

Of these stages in woman the first is said by Williams (638) to last twelve days, the second (the stage of premenstrual congestion) five days, the third (the actual period of menstruation) four days and the last seven days. According to other workers, however, the initial premenstrual changes begin much earlier than this, the period of quiescence being correspondingly shorter.

Shaw states that the earliest signs of activity in the intermenstrual endometrium become apparent about fourteen days after the beginning of the previous menstrual period. The surface epithelium becomes more columnar and then hypertrophied and dilated. Typical translucent areas appear behind the nuclei of the gland cells, which begin to secrete by the twentieth day. By this time also, the general hypertrophy has become much greater. After the twentieth day other changes become evident. Three layers can now be distinguished in the stroma :

- (a) A dense layer immediately below the surface epithelium; the cells are tightly packed together round the gland ducts in this area.
- (b) An œdematous layer which surrounds the glands and in which the cells are separated, but the capillaries dilated.
- (c) A basal layer in which the characteristic œdema and hypertrophy are absent.

These changes are accentuated until the 28th day when, if pregnancy fails to supervene, degeneration sets in. This consists essentially in the disorganization and disintegration of the glands of the superficial and middle zones of the endometrium. Coincidentally, blood is extravasated from the capillaries and, having formed small hæmatomata, finally reaches the lumen through the disintegrated epithelium. The extent to which the endometrium is destroyed and shed varies

very greatly in different individuals. Subsequently, the endometrium re-forms and returns to the resting stage.

The uterine changes in monkeys, as described by Heape, Corner, and other writers, are essentially similar. Heape divided the whole cycle into eight stages: 1. resting stage, 2. growth of stroma, 3. increase of vessels, 4. breakdown of vessels, 5. formation of lacunæ, 6. rupture of lacunæ, 7. formation of menstrual clot, 8. recuperation stage. Corner gives the following description of the menstruating uterus of *Macacus*: 'In general the menstrual process consists of an extravasation of blood in the more superficial part of the endometrium. This leads to the necrosis and collapse of the stroma and glands in the affected region, and here and there to the lifting away of small sheets of epithelium by formation of hæmatomata under them. It appears that most of the surface is denuded of epithelium; that portions of the glands may be lost, and that the stroma is necrosed and cast off to a depth of roughly one-fourth of the entire thickness of the endometrium. . . . As far as can be made out in these specimens regeneration of the epithelium is by growth from the glands and perhaps from the undisturbed islets of epithelium. The result is a smooth healing of the wound-surface with a single layer of very low epithelium.'

Information as to the time relations of the various phases of the uterine cycle in monkeys does not seem to be available, though the actual duration of menstruation (four to six days) is similar to that of the human, as is the total length of cycle.

*The ovarian cycle.* The difficulties in the way of obtaining suitable human material of known history, together with the untrustworthiness of the methods employed, led many of the earlier workers, such as Steinhaus (597), Jackson (314), Leopold (360) and Aveling (52), to doubt the existence of a regular ovarian cycle in man. This conclusion was supported by the erratic behaviour of the ovaries of Heape's monkeys. More recent work, however, and our general knowledge of mammalian reproduction, makes it necessary to suppose that a regular ovarian cycle exists in the normal human, as in lower mammals. In monkeys, on the other hand, under conditions of captivity in temperate climates, the ovarian cycle very often appears to be in abeyance. In both cases information as to the details of the



cycle is very meagre, the exact time of ovulation not being known, even in the human.

A tubal ovum has not yet been recovered in man and this method of determining the time of ovulation is not therefore available. Certain data are provided, however, by the history of the corpus luteum. The presence of newly-formed corpora lutea in the human ovary at fifteen to seventeen days after the beginning of the previous menstrual cycle led Meyer (459), Schröder (548) and Novak and TeLinde (475), among others, to conclude that ovulation takes place between these times, *i.e.* in the middle of the intermenstrual interval. Shaw's material enabled him to narrow the time down to between the thirteenth and seventeenth days. By the nineteenth day of the cycle the corpus luteum is mature, and it remains unaltered until the twenty-seventh day of the cycle. By the twenty-eighth day signs of degeneration, synchronizing with the onset of menstruation, have appeared, and eventually, some eight months later (560), a corpus albicans is produced.

In *Macacus*, ovulation (deduced from the recovery of tubal ova) has been found by Corner (123) to occur fourteen to fifteen days, and by Allen (9) ten to fourteen days after the onset of the previous menstruation. Apart from some notes by Allen on the corpora lutea, little further appears to be known of the ovarian cycle in monkeys.

*Vaginal and mammary cycles.* Both Allen and Corner have dealt with the vaginal cycle in *Macacus*. Their work makes it evident that, while slight changes occur in the vaginal epithelium and in the proportions of leucocytes in the smear, no striking cyclic changes occur such as are found in the mouse and rat. In other monkeys, notably *Papio* *sps.*, extraordinary swelling of the vulva occurs during the follicular phase.

In the human, Papanicolaou (486) has claimed to be able to detect all kinds of pathological conditions, as well as the normal cyclic stages of the uterus and ovaries, by changes in the vaginal smear. Dierks (160) has also described slight vaginal changes during the menstrual cycle. King (322), however, obtained negative results.

In monkeys, mammary changes do not appear to have been described during the ordinary menstrual cycle, though Heape

refers to enlargement of the nipples concurrent with menstruation. In the human, mammary hypertrophy during the immediate premenstrual phase is well known and has been considered in detail by Dieckmann (159).

*The interpretation of the Primate cycle.* Much controversy still exists as to the precise significance of the menstrual cycle in Primates. There can be little doubt that it is strictly comparable with the cycle in lower mammals, though the changes are greatly exaggerated at the uterine hæmorrhage stage. Apart from hypotheses which maintain that no correspondence exists between the menstrual cycle and the cycle in lower mammals, three different theories have been maintained at one time or other:

- (a) Most clinicians and embryologists have supposed that the premenstrual growth of the uterine endometrium is designed to facilitate the implantation of the embryo, and is thus a postœstrous phase, corresponding to the luteal or pseudo-pregnant phase of lower mammals. Menstruation on this view is supposed to represent solely the destruction of the prepared endometrium when the fertilized ovum has failed to materialize: in other words, to represent the abortion of an unwanted decidua. There is much evidence in favour of this view. Premenstrual congestion begins after ovulation, and the breakdown of the endometrium coincides with retrogressive changes in the corpora lutea. Degeneration of pseudo-pregnant congestion, sometimes accompanied by hæmorrhage, has been described in the rabbit by Hammond (264) and Ancel and Bouin (31), in the dog by Gerlinger (241), in the sow by Corner (122), and also occurs in the mouse.

Further evidence in support of this view is that X-ray sterilization stops the menstrual cycle in the human, whereas in lower mammals such treatment causes the disappearance of the luteal phase, but not of the follicular phase (see p. 177). Seitz and Wintz (557) even state that X-irradiation during the first half of the inter-menstrual period (*i.e.* early enough to stop the formation of the corpus luteum) leads to immediate

amenorrhœa, while irradiation during the second half (*i.e.* after the formation of the corpus luteum) does not interfere with the next menstruation.

The essential feature of the interpretation of the menstrual period as purely pseudo-pregnant degeneration is that preliminary activity of the corpus luteum is presupposed. This conflicts with the well-established fact that some humans (123, 314, 360), and many monkeys, have been known to menstruate without previous ovulation. Unless in such conditions menstruation is purely pathological (which its regularity makes very doubtful), its occurrence in non-ovulating animals is strong support of the second theory of menstruation.

- (b) From his studies on monkeys, Heape was led to suppose that menstruation was analogous with the proœstrous hæmorrhage which takes place in such animals as the dog, as described by Marshall and Jolly, and to a lesser extent in the cow and sheep. A similar view was initially held by Marshall. In general, the period of œstrus in mammals is characterized by destruction of the epithelium of the genital tract, such as the cornification of the vaginal epithelium in the rat, mouse, and guinea-pig. This merely represents the disintegration of a short proœstrous growth, and its identification with menstruation (as attempted by Stockard and Papanicolaou for the guinea-pig) is difficult. Such a point of view is strengthened, however, by the occurrence of menstruation without ovulation, and by Allen's (12) induction of menstruation in *Macacus* (a) by destruction of ripe follicles and (b) by injection into the ovariectomized animal of the œstrus-producing hormone.

The view that menstruation is proœstrous degeneration is difficult to reconcile with the time relations of the uterine and ovarian cycles. It is generally agreed that ovulation takes place about fourteen days after the beginning of the previous menstruation. In the lower mammals ovulation and œstrus are strictly

(h) SUMMARY TABLE  
TABLE 4.—CHIEF FEATURES OF THE ŒSTROUS CYCLE IN COMMON MAMMALS.

Species	Nature of Cycle	Ovulation	Length of Œstroous Cycle	Length of Pseudo-Pregnancy	Length of Gestation	Size of Litter	Cycle during Lactation	Age at first Œstrus
<i>Marsupials</i> <i>Dasyurus</i>	Monœstroous	Spontaneous	—	8-14 days	8-14 days	6 (pouch young)	Absent	
Opossum <i>Rodentia</i> Rat	Polyœstroous	"	27 days	11 days	13 days		Present	
	Polyœstroous	Spontaneous	5-6 days	12 days	22 days	6-8	Absent	10-11 weeks
Mouse Rabbit	" Persistent Œstrus	" After copulation	5-6 days —	12 days 14-16 days	19 days 30 days	5-7 5-9	" "	6-7 weeks 6 months
Guinea-pig <i>Ungulata</i> Cow	Polyœstroous	Spontaneous	16 days	—	62 days	2-3	Present	
Sheep	"	Spontaneous	3 weeks	—	9 months	1	Present	15 months
Goat	"	"	2-3 weeks	—	5 months	1-2	Absent	
Horse	"	"	2-3 weeks	—	5 months	1-2	Present	6 months
Pig	"	"	20-25 days	—	11 months	1	"	2 years
<i>Carnivora</i> Dog Ferret	"	"	3 weeks	—	4 months	10-15	"	6 months
	Monœstroous Persistent Œstrus	Spontaneous After copulation	— —	60 days 6 weeks	60 days 6 weeks	3-6 5-8	Absent Absent	10 months 7-10 months
<i>Primates</i> <i>Macacus</i> Man	Polyœstroous "	Spontaneous "	28 days "	— —	24 weeks 40 weeks	1 1	Variable	5 years 12-14 years



synchronized, and some extraordinary distortion must have taken place in the human cycle if fourteen days (*i.e.* half the total length of cycle) elapse between proœstrus and ovulation.

- (c) More recently, Marshall has suggested that menstruation represents both pseudo-pregnant and proœstrous degeneration telescoped into one (448). In animals such as the guinea-pig with a diœstrous cycle containing a luteal phase, the end of the latter (pseudo-pregnancy) is rapidly followed by proœstrus, and a further contraction of the cycle at this point might result in these stages becoming merged. This view of Primate menstruation is satisfactory in explaining many difficulties. It admits the essential pseudo-pregnant nature of the premenstrual growth of the uterus, and explains menstruation in non-ovulating animals by the supposition that the part of menstruation occurring under such conditions is the proœstrous stage only. Corner's (126) observation that menstruation without ovulation is not preceded by the typical premenstrual growth of the endometrium is therefore of great interest. Allen's (12) results can also be explained on the grounds that the rupture of large follicles and the cessation of injection of the œstrus-producing hormone precipitate the destruction of proœstrous growth.

Even this view, however, has the difficulty that the destruction of proœstrous growth will have ended some nine days before ovulation, and it does not explain such abnormal phenomena as menorrhagia associated with cystic follicles.

## CHAPTER V

### THE OVARY AS AN ORGAN OF INTERNAL SECRETION

THE synchronization found between development in the ovary and activity in the accessory reproductive organs immediately suggests that one controls the other, and it has become abundantly clear that the ovary is responsible for the growth and cyclic changes of the uterus, vagina, and mammary glands. Furthermore, the evidence shows that the nervous system plays a very minor part in this correlation, which is maintained by means of internal secretions. This view is based primarily upon ovariectomy and transplantation experiments, and in a lesser degree upon cases of abnormal sexuality. Such evidence in itself is not quite complete, and the ultimate proof (extraction of the active substances themselves from the ovary) is only just being obtained after a long period of very indifferent work.

The effects of ovariectomy and ovarian transplantation, and the earlier experiments on the use of ovarian preparations, are discussed below.

#### (a) EFFECTS OF OVARIECTOMY

Gonadectomy in both mammals and birds leads to the development of a type which is intermediate between the two sexes, and which may conveniently be regarded as approximating to the neutral embryonic condition. In birds this neutral type tends to resemble superficially the male (the homozygous sex).

In birds, ovariectomy still leaves intact the rudimentary right gonad, which may subsequently develop into testis tissue. This results in sex-reversal, which should not be confused with

the effects of complete gonadectomy. The same probably applies to the assumption of male characters which may happen in mammals when the ovary is interfered with by

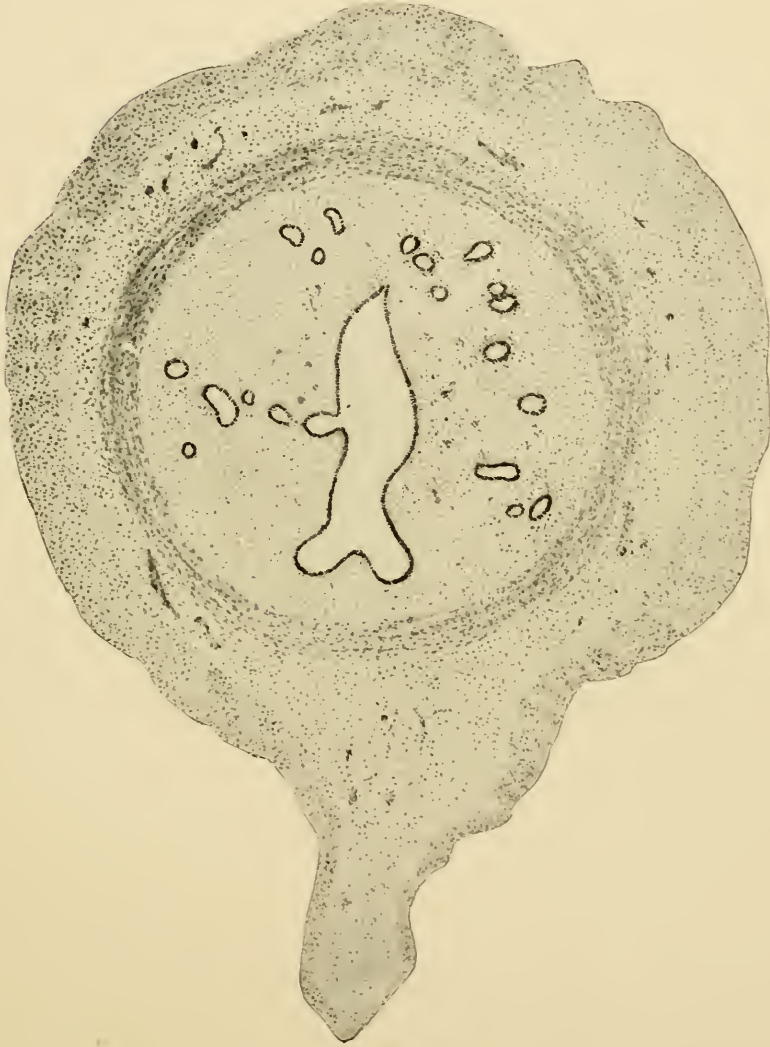


FIG. 36.—NORMAL UTERUS OF RAT.  
(From Marshall and Jolly.)

malignant growth, or bacterial action, or at the menopause. Complete gonadectomy of the female mammal does not lead to the assumption of male secondary characters.

*Effects on secondary characters.* As indicated above, owing to the negative nature of the female secondary sexual characters, the superficial effects of ovariectomy are not as obvious as those

of castration. Certain effects, however, unconnected with the accessory organs, do follow ovariectomy, particularly of the immature female. These include:

- (a) The subcutaneous fat which is laid down at puberty in the normal female fails to appear, and, subsequently, adipose tissue is laid down indiscriminately.
- (b) The long bones undergo increased growth owing to the non-ossification of the epiphyses.
- (c) In correlation with the increased growth of the skeleton, a greater weight is attained. Experimental work on this has been performed by Wang, Richter, and Guttmacher (630).
- (d) The sex instinct is suppressed, together with such sex reflexes as the 'tail erection' of the female rodent before copulation. With the suppression of the sex instinct comes a general state of lethargy. Advantage is taken of this fact in the spaying of farm stock before fattening for market.
- (e) According to Loewy and Richter (424) the basal metabolism falls after ovariectomy. Blair Bell (56) reports decrease in calcium excretion, increase in phosphorus excretion, and no change in nitrogen metabolism after ovariectomy.
- (f) Much confusion exists as to the effect of ovariectomy on the other ductless glands. Biach and Hulses (64) state that ovariectomy of the young animal causes atrophy of the pineal. This has not been confirmed. Hammond (262) and Blair Bell (56) found hypertrophy and hyperactivity of the pituitary after ovariectomy. Considerable hypertrophy of the thyroid is known to occur during various phases of the sexual cycle, and Blair Bell reports hyperactivity after ovariectomy. According to the same author, an increase in the zona reticularis of the adrenal is found. According to Masui and Tamura (457), Miller (460), and Deanesly (154), sex dimorphism is found in the adrenal cortex of the mouse. Castration causes changes in the male cortex which obliterate the sex difference, but ovariectomy produces no effect on the female gland.



It will be noted that many of these effects of ovariectomy are general results of de-sexing the animal and occur after castration of the male. Similar effects are also found in chronic insufficiency brought on by delayed development or hypo-function of the gonad.

*Effects on the accessory organs.* Ovariectomy causes profound changes in the accessory organs of reproduction, the effect being more striking when the ovaries are removed before puberty. The operation at this time results in complete failure of the organs to undergo their normal development, and in non-

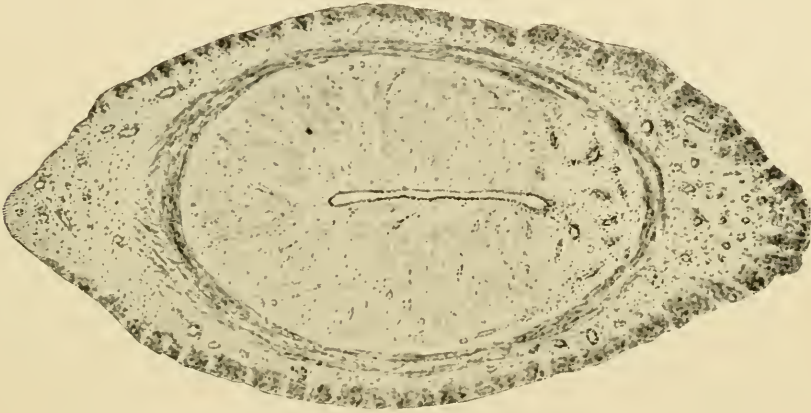


FIG. 37.—UTERUS OF RAT AFTER OVARIECTOMY, SHOWING DEGENERATIVE CHANGES.

(From Marshall and Jolly.)

appearance of the cyclic changes of œstrus. Post-pubertal ovariectomy leads immediately (but see p. 137) to the stoppage of all cyclic activity in the uterus, vagina, and mammary glands, but the actual degeneration of the organs is slow, and the degree of atrophy found at autopsy is usually proportional to the time after operation. The general effects on the accessory organs are suggestive of the changes occurring at the menopause, and ovariectomy is often spoken of clinically as producing an 'artificial menopause.'

Carmichael and Marshall (114) have reported extensive experiments on the adult rabbit. Six months after ovariectomy the uterine stroma had entirely degenerated, the glands, mucosa, and muscle layers having undergone atrophy. Degeneration had also extended to the Fallopian tubes. Similar

results were obtained by Bucura (109) on the rabbit, by Marshall and Jolly (452, 453) on rats, by Tandler and Keller (609) on cows, and by Halban (260) on guinea-pigs. This last author also states that the mammary gland fails to develop after pre-pubertal ovariectomy.

Ovariectomy in the human leads to the degeneration of uterus, vagina, and mammary glands, or, if the operation is pre-pubertal, to the non-development of these organs. In the

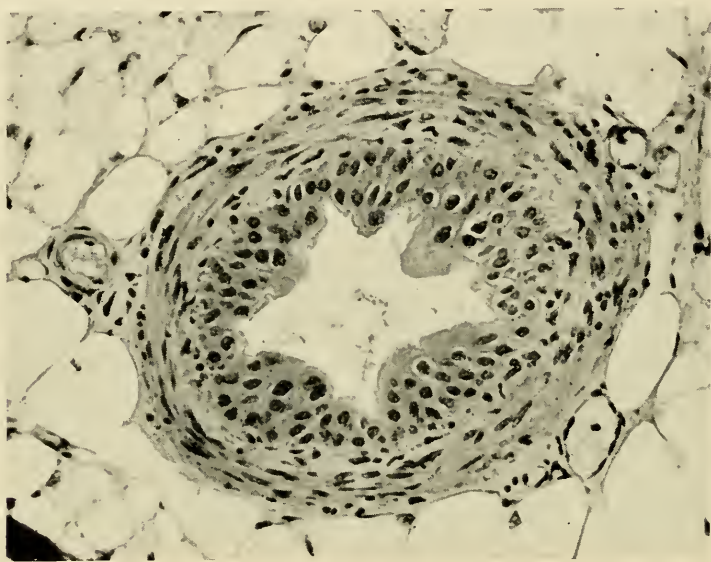


FIG. 38.—UTERUS OF MOUSE AFTER PRE-PUBERTAL OVARIECTOMY, SHOWING RUDIMENTARY CONDITION.

case of the mammary glands, however, it is necessary to make a qualification; there is some evidence that if the gland is secreting at the time of the operation, the secretion may be prolonged.

In the mouse (508) the effects of pre-pubertal ovariectomy (at three weeks old) are remarkable. Two months after the operation the uterus is not only undeveloped, but has actually degenerated from the pre-pubertal state, being only about  $\cdot 1$  mm. in diameter. Microscopically it consists of a 'stroma,' three to four cells thick, surrounded by a few layers of muscle fibres. The changes in the vagina are equally definite. Though about the same size as the pre-pubertal organ, the vagina after ovariectomy is collapsed, the component layers are atrophic, and the lumen is blocked by debris. No effects on

the clitoris are produced by ovariectomy of the immature mouse, a fact which emphasizes the vestigial nature of the clitoris in this animal.

These effects of ovariectomy are now almost universally ascribed to the removal of ovarian influence. Early workers, such as Hofmeier (308), were inclined to attribute the effect to



FIG. 39.—VAGINA OF MOUSE AFTER PRE-PUBERTAL OVARIECTOMY, SHOWING RUDIMENTARY CONDITION.

*d.l.* debris in lumen; *w.r.* wall of rectum.

disturbance of the vascular supply of the uterus by the operation, or to interference with the nervous connections, as suggested by Sokoloff (588). Many workers, however, have taken specific precautions against such possible effects, and, in any case, the operation of ovariectomy can hardly interfere with the vascular supply of the mammary glands. Similarly, another early hypothesis, that hysterectomy causes atrophy of the ovary, is now untenable (114).

#### (b) OVARIAN TRANSPLANTATION

The facts discussed in the previous section make it clear that the development and maintenance of the accessory organs of



reproduction, together with the occurrence of the changes characteristic of the œstrous cycle, are dependent upon the presence of the ovary. It is now necessary to consider the next link in the chain of evidence that the ovary is an organ of internal secretion. So far as the effects of ovariectomy are concerned, the ovarian control of the accessory organs might be a nervous one, and it is necessary to show that the nervous connections of the ovary may be entirely destroyed without adverse effects upon the accessory organs, before this hypothesis can be dismissed. Such a demonstration is provided by the transplantation of the ovaries to a different site in the body.

*Histology of the ovarian graft.* The technique of grafting ovaries has been described at length by many authors, including Marshall and Jolly (452) and Lipschütz (378). The usual site of transplantation is the abdominal wall, the peritoneum, or the kidney. Grafts readily 'take' and survive if the ovary is from the same individual (homoplastic graft), but survival is less satisfactory when the ovary is from another individual even of the same species (heteroplastic graft). Tissue from an animal of a different species rarely survives for long. Many workers have considered the histology of grafted tissue, especially that of the ovarian graft into the male. Amongst these may be mentioned Ribbert (534), Marshall and Jolly (452, 453), Schultz (551), Steinach (591), Athias (50, 51), Moore (462), Pettinari (515), Sand (542), Lipschütz (378), and Tamura (607).

The first effect of transplantation of the ovary is that the larger follicles tend to undergo atresia, and the corpora lutea degenerate. If good vascular connections have been established, the graft in the female undergoes growth, and the cyclic changes characteristic of the normal ovary take place. If the graft has a free surface, ovulation occurs, and true corpora lutea are formed. Pregnancy from an ovarian graft has been recorded by certain authors, including Morris (465) and Grigorieff (250). Marshall and Jolly (453) state that homoplastic grafts in the rat were found to be normal and to contain corpora lutea after fourteen months, while heteroplastic grafts were normal after six months. All workers agree, however, that follicular atresia is more common in the grafted ovary than in the normal;



this leads to the production of lutein tissue, and to the appearance of large amounts of interstitial tissue in species



FIG. 40.—OVARY OF RAT AFTER TRANSPLANTATION TO THE PERITONEUM, SHOWING CORPORA LUTEA AND SMALL FOLLICLE.

(From Marshall and Jolly.)

where it originates from atretic follicles. Cystic follicles are also common in the ovarian graft.

Ovarian tissue grafted into the male animal differs from that grafted into the female, in that all follicles appear to become

cystic or atretic. Only Pettinari (514) has observed true corpora lutea in the ovary grafted into the male.

Male guinea-pigs with such a graft become 'hyperfeminized' and secrete milk. Lipschütz attributes this effect to the cystic follicles, and likens it to a condition of persistent œstrus. This author states, however, that the atretic follicles form luteal and interstitial cells. Since similar luteal cells formed in other experimental ways (see p. 186) can be shown to have their normal function, it is doubtful whether the mammary effects found in experimental hermaphroditism are brought about by follicular or luteal action.

*Effects of transplantation.* Many workers have reported the effects of ovarian transplantation, including Knauer (325), Ribbert (534), and Limon (364) on the rabbit, Halban (260) on the guinea-pig, and Marshall and Jolly (452) on the rat.

As a result of this work, it has become abundantly clear that the successful transplantation of the ovaries from their normal position to some other site in the body never results in the changes typical of ovariectomy.

Moreover, grafts from another individual into an ovariectomized female will prevent the atrophy which would otherwise take place. It should also be emphasized that under favourable conditions œstrous changes can occur in the ovariectomized animal with an ovarian graft. This means that, provided vascular connections exist, the ovary can control the growth and periodicity of the secondary organs from anywhere in the body, and hence that no nervous mechanism is concerned.

It does not seem to have been recorded whether the ovarian graft will maintain the œstrous cycle after atresia of the follicles, but it is certain that such a graft can maintain the ordinary nutrition of the uterus. The ovarian factor concerned is thus independent of the follicles. This conclusion has been supported by the grafting of ovarian medulla only (which is free of large follicles) as reported by Blair Bell (56), and by X-ray elimination of the follicles (see p. 143) in the young animal.

Further evidence that the correlation between the ovary and the accessory organs is non-nervous in nature may be mentioned. Instead of severing the nervous connection by transplantation of the ovary, it is possible to perform the converse experiment and

graft portions of the accessory organ to another site. Thus uterine mucosa has been transplanted by Loeb (380) and



FIG. 41.—UTERUS OF RAT AFTER TRANSPLANTATION OF THE OVARY.

(From Marshall and Jolly.)

The uterus is normal (*cf.* fig. 36).

mammary tissue by Ribbert (534). In both cases the normal ovarian influence was manifest on the graft.



## (c) INTERSEXUALITY

The evidence that the ovary performs its functions by other than nervous means may be supported by our knowledge of sexual abnormality—both spontaneous and experimental. The information relating to experimental intersexuality in mammals has largely been summarized in the preceding section dealing with the ovarian graft, but instances of spontaneous intersexuality provide striking demonstrations of the endocrine action of ovarian tissue. A full discussion of these abnormalities is given by Crew (149), Goldschmidt (248) and Brambell (81).

## (d) ADMINISTRATION OF OVARIAN PREPARATIONS

The data discussed above show that the ovary controls the development and periodicity of the accessory organs, and that no nervous mechanism is involved. It is now necessary to consider what progress has been made towards the final proof that the control is endocrine in nature, *i.e.* towards the preparation of the active substances.

Brown-Sequard (107) appears to have originated the use of ovarian preparations in cases of ovarian deficiency. Following his classic experiments, many workers reported results of the same nature, including Loewy and Richter (424), Neuman and Vas (473), and Camboulas (113).

The ovarian tissue was usually administered by mouth, fresh or dried, or simple extracts were eaten or injected. The criteria of ovarian activity used by these early workers were remarkably varied, ranging from obscure metabolic effects to psychological changes.

Most of the experiments were clinical, and the results were as varied as the modes of administration and the criteria of activity. Various subsequent authors have pointed out the probability that any results obtained could have been produced with any tissue extract. These early workers, however, though producing few results of value, yet laid the foundations of the direct study of ovarian secretion.

Later, the fundamental need for accurate criteria of activity began to be recognized. In the present state of our knowledge



such criteria must be the simulation in the ovariectomized animal of ovarian influence, *i.e.* the induction of development or change in the accessory organs after removal of the ovaries.

The prevention of ovariectomy effects by means of extracts was attempted in 1900 by Jentzer and Beuttner (315) and in 1907 by Bucura (108), but no very definite results were obtained.

Since then a vast amount of work has been carried out with ovarian extracts, interest largely centring on the production of the changes characteristic of œstrus, and more recently on the substitution of luteal activity. These experiments, dealt with in Chapters VI and X, have completed the chain of evidence that the ovary carries out its control of the accessory organs by means of internal secretions.

#### (e) THE INTERNAL SECRETION COMPLEX OF THE OVARY

The work discussed in the preceding chapters shows that the ovary performs three general functions by means of endocrine activity:

- (a) Control of early development of the accessory organs and secondary sexual characters.
- (b) Control of the cyclic changes characteristic of œstrus in the accessory organs.
- (c) Control of the changes occurring during pregnancy.

The question now arises as to whether one internal secretion can perform all of such varied functions. Marshall originally postulated that three ovarian hormones exist, one connected with each of the above functions. Marshall also supposed that the Graafian follicle probably produced the hormone associated with œstrus, and the corpus luteum the one associated with the changes of the luteal phase of the cycle (pregnancy and pseudo-pregnancy). This conception has been accepted in essence by many authorities, including Loeb (400), Corner (125), Evans (425), and Courrier (137), the last of whom says: 'Nous pouvons affirmer que l'ovaire secrète au moins deux hormones, l'hormone folliculaire et l'hormone luteinique.'

Recently, however, a tendency has arisen to regard the

œstrus-producing hormone (see p. 121) as the only ovarian hormone. This view is based mainly on the fact that injection of this substance will cause the immature uterus and vagina to show œstrous symptoms, and on the comparative failure up to the present to obtain extracts which will substitute the presence of the corpus luteum.

Active extracts of luteal tissue are now, however, being prepared, and it may be said with some confidence that at least two ovarian hormones exist. Probably three, as originally suggested by Marshall, will eventually be demonstrated.



## CHAPTER VI

### THE ŒSTRUS-PRODUCING HORMONE

#### (a) HISTORY OF PREPARATION

THE history of the preparation of the œstrus-producing principle of the ovary may be divided into three periods. The first, beginning about 1912, comprises all the early work on the extraction of the ovary. At this time workers were largely concerned with the preparation of ovarian extracts which would have some action on the reproductive organs, especially in the ovariectomized animal. The majority of the preparations appear to have contained the œstrus-producing hormone, and the positive effects reported probably depended upon its presence in the extracts. The second period may be said to have begun with the work of Allen and Doisy, who adopted a specific test for œstrus-producing activity and elaborated an easy technique for the examination of results in the live animal. The œstrous cornification of the vagina of rats and mice, which can be detected by means of vaginal smearing, made both these advances possible. The methods of extraction used by Allen and Doisy and by their immediate followers in this field were essentially the same as those of earlier authors. The third period begins with the preparation of œstrus-producing extracts in a water-soluble or pseudo-water-soluble form. Progress may thus be said to have alternated between advance in the technique of testing and advance in the methods of preparation.

*Early work on ovarian extracts.* The first ovarian extracts appear to have been designed to prevent the atrophy of the uterus which follows double ovariectomy. Jentzer and Beuttner (315) and later Bucura (109) were among the first to attempt this experimentally, but their saline extracts were not effective. In 1906, Marshall and Jolly (451) produced transient

œstrous changes in the anœstrous bitch by the injection of saline extracts of ovaries, but Sonnenberg (589) in 1907 failed to cause œstrus by the injection of liquor folliculi. In 1912 a variety of workers published the results of experiments on the effects of ovarian extracts on the accessory organs. Adler (2) claimed to have produced œstrous changes in virgin rabbits by the use of aqueous extracts of the whole ovary. In the same year Iscovesco (310-3) was able to extract, by the use of fat solvents, a substance which caused rapid hypertrophy of the uteri of adult animals, the uteri of injected animals being three to four times as heavy as those of controls. To Fellner (191-2), however, falls the credit for the first organized attempt to analyse the effects of extracts of various reproductive organs on the normal and ovariectomized animal. Fellner appears to have aimed at causing changes characteristic of pregnancy in the organs of the ovariectomized animal. His results, however, showed fairly clearly that œstrous changes were produced. Extracts of whole ovary, of corpora lutea, of ovaries without corpora lutea, as well as placental extracts were used, and the effects of these extracts on the uterus, vagina and mammary glands were investigated. The real advance made by Fellner lay, firstly, in the use of ovariectomized animals as test animals, and, secondly, in the careful examination of the effects produced. Fellner stated that his active substance obtained from placentae, ovaries without corpora lutea, and Graafian follicles, was soluble in alcohol, ether, and acetone, and was therefore probably of a lipid nature.

In the following year Okinschitz (481) stated that the subcutaneous injection of extracts into ovariectomized rabbits retarded the atrophy of the uterus. Extracts of corpora lutea were not found to have this effect. At about the same time Seitz, Wintz, and Fingerhut (558) claimed to have prepared from the corpus luteum two different substances, one of which promoted menstruation, while the other inhibited it. Herrmann (291) and Herrmann and Fraenkel (294), who used immature rabbits as test animals, emphasized the presence of the œstrus-producing substance in the placenta. These authors give the following directions for the preparation of extracts. The tissue is minced and extracted twice with alcohol, the two extracts



being combined. The alcoholic extract is then evaporated to dryness and the residue extracted with ether; the phosphatides are precipitated from the ethereal solution with acetone. The acetone extract contains the active principle. Since 1915, Frank and his collaborators (219-231) have reported a series of observations agreeing roughly with those of Herrmann and Fraenkel. These authors emphasize the presence of the œstrus-producing substance in both placentae and corpora lutea. The extracts were tested upon immature rabbits, and later upon rats. Similar observations have been reported by Aschner (41). Wintz (640), and Seaborn and Champy (553) have used untreated liquor folliculi for injection purposes with positive results. Fellner (193-8) has amplified his original observations from time to time.

*Recent work on the œstrus-producing hormone.* The application of the vaginal smear technique by Allen and Doisy to the testing of ovarian extracts made possible a great advance in the whole study of the internal secretions of the ovary. Their extracts were made in much the same way as those of previous workers, but various elaborations were introduced. Allen and Doisy originally used liquor folliculi from cow or pig as their starting material. The liquor folliculi was obtained by aspiration with a hypodermic needle from the larger follicles. The fluid was mixed with twice its volume of 95% alcohol and allowed to stand until the precipitated proteins had coagulated. The mixture was then filtered, and the residue again extracted with alcohol to remove the fraction of the hormone adsorbed by the proteins. The two alcoholic filtrates were then mixed and evaporated down to a watery residue, which was then extracted with ether. The ether-soluble fraction was extracted with acetone in order to leave behind the phosphatides. The acetone-soluble fraction was re-extracted with 95% alcohol, by which means much of the inert fat was eliminated. Further purification was obtained by precipitating cholesterol with methyl alcohol at 0° C. A similar process with certain modifications in the early stages was used for the extraction of solid tissue. From their initial investigations, Allen and Doisy concluded that an extract could be obtained which would produce all the normal extra-ovarian symptoms of œstrus in the ovariec-

tomized rat or mouse. These authors considered that liquor folliculi was the essential source of the hormone and that the small yields obtained by them from residual ovarian tissue were due to incomplete removal of the follicular fluid. They found that no œstrus-producing substance could be obtained from the solid corpora lutea of lower mammals, but they were later able to demonstrate its presence in the human corpus luteum during the early stages of its development.

The work of Allen and Doisy and their collaborators has stimulated a great deal of interest in the œstrus-producing hormone, and many authors have since amplified and confirmed their chief results. Frank and his collaborators have prepared the hormone from ovaries, placentae, and corpora lutea, and consider that only one ovarian hormone is present. They have also described a modified technique for the treatment of small quantities of blood (227) by ether extraction after dehydration in anhydrous  $\text{Na}_2\text{SO}_4$ . Courrier (133-9), working on the guinea-pig and rabbit, has described the effects of injection of crude liquor folliculi and of extracts. Other workers in the same field include Papanicolaou (485), Loeb and his collaborators (404-5), Brouha and Simmonett (89-93), Dodds and his collaborators (158), Zondek and Ascheim (646-655), Bugbee and Simond (110-112), Laqueur and his collaborators (343-356), Lipschütz (373), Loewe (407-419), and Parkes and Bellerby (503-7). These workers have been concerned primarily with studying the distribution of the hormone and with elaborating methods of preparation.

*The preparation in a water-soluble form.* The work immediately following that of Allen and Doisy was largely based on similar methods of extraction, namely, upon the assumption that the hormone was either fat-soluble or at least closely associated with fats. This assumption implies the use of organic fat solvents in the preparation of extracts. The use of these solvents, however, inevitably brings into solution a large amount of inert material, the presence of which makes such extracts very unsuitable for injection into sensitive animals, particularly the human subject. Various workers, therefore, have endeavoured to obtain active extracts by other methods, or to separate the active material from the oily product. In

general, the recent water-soluble extracts have been prepared (a) by initial aqueous extraction, and (b) by treatment of the fat extract with a view to freeing the active material.

The first successful attempt appears to have been made by Zondek and Brahn (661), who were able to detach the hormone from the unsaponifiable matter of the oil by the use of acid. A protein-free dialysable product, giving a clear solution in water and having a high activity could be obtained. More recently Zondek (648) has described a modification of this process for securing the hormone from urine. The essential feature of this modification is that the urine is extracted directly with ether or chloroform, the use of alcohol being avoided.

Soon after the work of Zondek and Brahn, Laqueur and co-workers (355, 356) reported the preparation of active aqueous solutions direct from liquor folliculi, without the intermediate production of an oily extract. Colloidal iron was added to diluted follicular fluid, and the mixture centrifuged. This yielded a turbid yellow fluid, which on partial evaporation at low temperatures yielded a clear fluid containing the hormone and a precipitate. The weight of the unit obtained by this process was very great, about 50 mgms., and for this reason Laqueur re-introduced the use of organic solvents. Later, he reported (355) the preparation of 'menformon' from placenta by initial boiling with benzene, extraction of the benzene extract with alcohol, and treatment with dilute HCl. After centrifuging or filtering, a very active water-clear solution is obtained. The process appears to be similar in principle to that of Zondek and Brahn, but it has been much criticised. The yields obtained are said to be 600-700 m.u. from one placenta. Laqueur stipulates a minimum purity of 1 m.u. = 1 mgm. before the substance may be called 'menformon.' Glimm and Wadehn (245) obtained an aqueous solution by dissolving the oil in 40% methyl alcohol and evaporating down in vacuo. Activity was found in the aqueous residue. They (246) mention the partial solubility of the active fraction of purified oily extracts in lime water, while Loewe (410) also discusses the possibilities of obtaining the material in water solution.

Dodds and co-workers, after an indifferently successful

attempt to produce an active picrate extract (156-7), have very recently reported the production of a water-soluble extract by a new method. These authors (5) use an initial baryta saponification of the placenta. After filtering through 'jean,' the filtrate is extracted with butyl alcohol, which is evaporated down and the residue taken up in hot water. This is then extracted with ether, and again evaporated down. After dissolving in alcohol, and re-saponification with barium hydroxide, the barium can be removed to leave an aqueous solution. The yields obtained are satisfactory (about 200 r.u.k.). The weight of the unit is about .01 mgm. and some 10-50 units per c.c. are obtained. This process, and that of Zondek and Brahn, may be relied upon to give consistent results. Steinach and co-workers (593) report the preparation of a highly active aqueous extract, in which the end product is said to have an activity of 500 m.u. per c.c.

*Nomenclature.* To refer to the œstrus-producing hormone of the ovary as 'the ovarian hormone' or as 'the female sex hormone' is to pre-suggest that only one exists. 'Feminin' used by Glimm and Wadehn and by Fellner is open to the same criticism. The term 'follicular hormone,' though showing its connection with the follicular phase of the cycle, is misleading in so far as it suggests elaboration only by the Graafian follicle. 'Folliculin,' used by Courrier and others, suffers from the same disadvantage. 'Menformon' used by Laqueur, appears to be meaningless, but 'thelykinin,' suggested by Loewe is more suitable. The latter author uses the term 'thelytropine' to include both the œstrus-producing hormone and the luteal hormone, which he terms 'thelystasin.' Blotevogel, Dohrn and Poll use 'tokokinin' to include all sex hormones. A name is clearly advantageous for descriptive purposes, and since the substance in question has not adequately been shown to produce any features other than those characteristic of œstrus, the name 'œstrin' (503) will be used here.

#### (b) CHEMICAL PROPERTIES

The major part of the work on the chemical properties of œstrin was carried out with the fat-soluble extracts, which



were rarely obtained purer than  $\cdot 1$  mgm. per mouse unit. Since the weight of the mouse unit can now be reduced to  $\cdot 001$  mgm. and even lower, at least 99% of the solid matter of these fat extracts must have been inert material. Probably the figure is at least 99.9%. This fact quite invalidates such chemical analyses as those of Fraenkel and Fonda (218), Hartmann (280), and Hartmann and Isler (281), regarding the nature of the hormone itself. Even now it is doubtful whether the positive properties of active extracts give any information about the hormone, and even negative reactions, which might give information, may be greatly influenced by the nature and amount of the impurities. To obtain 5 gms. of material of the present maximum purity for chemical work would involve the preparation of fifty million mouse units, equivalent to 50-100 tons of ovaries, placentae, or urine of pregnancy. Furthermore, it should be emphasized that until methods of quantitative estimation of activity become much more accurate, it is impossible to say whether or not the hormone shows complete resistance to such procedures as saponification.

*Solubility.* There can be little doubt that the active substance is readily soluble in fat solvents such as alcohol, ether and acetone, benzene, chloroform, etc. In petroleum ether, however, the solubility is comparatively low, as shown by Ralls, Jordan and Doisy (524), and Glimm and Wadehn (246), who have used this characteristic in the purification of the hormone.

Its essential solubility in organic fat solvents has been emphasized by many workers, including Herrmann, Fellner, and Allen and Doisy, but it has been shown that under certain conditions the hormone can be secured in an aqueous solution. A number of workers (see p. 87) have obtained perfectly clear aqueous preparations with a high activity. These are probably true solutions rather than colloidal suspensions, because all these workers have reported that the active material can be dialysed, and Zondek and Brahn report the absence of a Tyndall phenomenon. Slotta (570) has pointed out, however, that the solubility must be very slight, not more than  $\cdot 005\%$ . In any case, it is far less soluble in water than in fat solvents, because it can be re-extracted from aqueous preparations by ether.

*Thermostability.* The active substance is resistant to

comparatively high temperatures, though the exact temperature at which destruction takes place appears to vary with the nature of the impurities. Laqueur, Hart, and de Jongh (346) found that their less pure preparations resisted up to  $360^{\circ}\text{C}.$ , but that the purified aqueous solution was inactivated at  $170^{\circ}\text{C}.$

*Chemical constitution.* Ralls, Jordan, and Doisy (524) found that phosphorus was not present in active extracts, while Laqueur, Hart, de Jongh, and Wijsenbeek (356) failed to find nitrogen. This was confirmed by Wadehn (627), who, in addition, failed to find sulphur, and alcohol and amino groups. Laqueur, Hart, and de Jongh (355) report the absence of histidine and sulphur.

*Colour reactions.* Laqueur, Hart and de Jongh (355) obtained negative results with the Biuret and Diazo tests. Laqueur (344), Dodds and co-workers (158) and Frank and his co-workers (230) have obtained negative results with the Liebermann and Burchard-Salkowski tests for cholesterol.

*Oxidation, bromination, hydrogenation.* Œstrin is readily destroyed by oxidation, undergoing slow inactivation from atmospheric oxygen, and rapid destruction by oxidizing agents such as potassium permanganate (166, 524) and phosphorus pentachloride (230). Ralls, Jordan, and Doisy (524) state that the hormone survives hydrogenation, but according to Giesy (see Allen and Doisy, 21), it is inactivated by bromination. A similar conclusion has been reached by Doisy and co-workers (166, 524), and Frank and co-workers (230). This susceptibility to bromination does not necessarily indicate the presence of a double bond.

*Resistance to enzymes.* Œstrin resists the lipases of plants (166), of the pancreas, and of the liver (167). Laqueur, Hart, and de Jongh (346) confirmed these facts on their water-soluble products, which also showed resistance to pepsin.

*Saponification.* Doisy, Ralls, Allen, and Johnston (167) reported resistance to saponification by sodium ethylate and this has been confirmed by many workers. Laqueur, Hart, and de Jongh (344) boiled purified preparations with 26% KOH and 26%  $\text{H}_2\text{SO}_4$  without causing destruction, and the same authors (346) showed that it resisted 10% KOH up to  $170^{\circ}\text{C}.$  Other

agents to which it is resistant include NaOH (230), and sodium methylate and butylate (166, 167). Similar results have been reported by Payne, Peenan, and Cartland (511). Mild saponification is now a part of many routine methods of preparing the hormone.

*Volatility and sublimation.* The hormone is non-volatile and does not sublime (346).

*Adsorption.* The hormone is very readily adsorbed. Thus the precipitation of the proteins from liquor folliculi removes much of the hormone as a result of adsorption. According to Laqueur (357), the hormone is readily adsorbed by dialysing membranes and by filter paper.

*Action of ultra-violet light.* Jordan and Doisy (317) report that preparations of œstrin in petroleum ether, or in solutions containing fluorescent substances, lose their activity on exposure to light. The destruction is more rapid in ultra-violet light or direct sunlight, but may also occur in diffuse light.

### (c) ADMINISTRATION

*Tissue implants.* For testing pieces of tissue too small to extract, and also to save chemical treatment, Zondek and Aschheim (650) have used a method of intra-muscular implantation. This technique consists in the implantation of a small piece of the tissue to be tested into a muscle. No graft is formed, the implanted tissue is reabsorbed, and with it any active substances present. The procedure is similar in principle to the subcutaneous injection of tissue macerated in saline, which is usually the first step in investigating the activity of a tissue. The technique has obvious limitations, impracticability of repeated administration, for instance, but valuable results have been obtained by the method, notably the discovery of the ovary stimulating properties of the anterior pituitary body.

*Oral administration.* It is generally agreed that amounts of œstrin known to be active by other means of administration have no activity by mouth. Five tests were made by Allen (24) of administration by stomach tube, and uniformly negative results were found. Loewe, Lange, and Faure (409) report that large doses up to twenty times the normal amount are required

to produce a reaction by mouth, while Laqueur, Hart, and de Jongh (349) found a hundred times as much to be necessary. Hannan (268) states that sixty times as much is needed orally as subcutaneously. The fate of the hormone in the alimentary canal does not seem to be known. It was naturally thought that its destruction was due to the digestive enzymes, but this expectation does not seem to have been confirmed. Also, the degrees of acidity and alkalinity normally found in the alimentary canal can hardly be sufficient to destroy the hormone. It is possible that the active substance passes through the tract without being absorbed, but it has been found impossible to detect the hormone in the faeces after heavy administration by mouth.

*Subcutaneous injection.* Preparations are usually administered by subcutaneous injection, but the behaviour of the preparation after such administration varies very greatly according to the chemical nature of the extract. Preparations containing much cholesterol invariably cause granulation and subsequent sloughing. In these cases where much inactive matter is present, the absorption of the active substance can hardly be very complete, and no accurate assay can be carried out where this complication occurs. The fact that a large injection of a relatively impure preparation will produce œstrous symptoms for more than fourteen days seems to show that absorption under such conditions is very slow. Since the duration of œstrous symptoms following injection probably depends largely upon the rate of absorption, and therefore upon the purity of the extract, it cannot be of any value in assaying the activity of an extract.

*Intra-peritoneal injection.* Intra-peritoneal administration has not been found to have any advantages over subcutaneous injection. Allen and Doisy (24) and Coward and Burn (146) were unable to detect greater efficiency, and Evans and Burr (184) even report an increased activity from subcutaneous injection.

*Intra-venous injection.* This type of administration has hardly been possible with crude fat-soluble extracts, but the comparative purity of the recent water-soluble preparations may make administration direct into the blood stream a



possibility. Under such conditions, however, frequent injections would be required to keep up the continuous action on the reproductive organs which is necessary for a positive result.

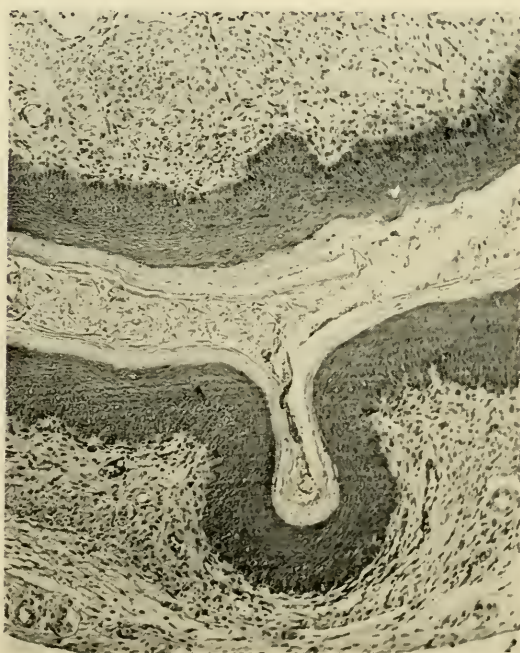
(d) TEST-OBJECTS

Any biological test of œstrus-producing activity must clearly depend on the production of œstrous changes when they would not otherwise occur. Immature, anœstrous, diœstrous, or ovariectomized animals must therefore be used. Of these, all except the last are unsuitable, though they have been used, because it is impossible to eliminate entirely the possibility of spontaneous ovarian activity. Ovariectomized animals are, therefore, highly desirable and are generally used. The œstrous changes which can be used as criteria of activity are various. Changes in the vulva are very indefinite (except in the ferret and certain Primates), while the œstrous growth of the mammary gland is not yet known sufficiently to be of value. In most animals the uterine changes are quite distinct, but autopsy is required for every test. The two desirable conditions, that the change shall be abrupt, and discernible in the intact animal, are best satisfied by using the vaginal reaction of the mouse, rat, or guinea-pig.

*Rabbit uterus.* In seeking some means of testing the activity of ovarian extracts on the accessory reproductive organs, the early workers used immature animals, usually the rabbit, as test animals, and considered hypertrophy of the uterus as a positive result. Subsequently, ovariectomized animals were used for the same purpose. As work on the extraction of ovaries became concentrated on the œstrus-producing hormone, hypertrophy of the uterus was considered as a sign of œstrus-producing activity. This test, however, cannot be said to be ideal because of (a) the uncertainty of the nature of the reaction unless histological examination is made; (b) the necessity for autopsy at every test; (c) the lack of an end-point in a positive reaction; (d) the difficulty of arriving at time relations; (e) the large amount of active material required; (f) the unsuitability for quantitative work. Uhlmann (620) gives some methods of assessing the intensity of the reaction.



(a)



(b)

FIG. 42.—UTERUS AND VAGINA OF OVARIECTOMIZED MOUSE INJECTED WITH ESTRIN, SHOWING THE DIFFERENCE IN THRESHOLD VALUE FOR THE TWO ORGANS, (a) uterus with no symptoms of œstrus, (b) vagina with typical œstrous cornification.

*Vaginal reaction.* The discovery by Stockard and Papanicolaou that the vaginal changes in the rodent could be used to determine the œstrous cycle in the intact animal has made possible a very great advance in the technique of testing for œstrus-producing activity. This technique now consists of injection of the ovariectomized test animal, and examination for the appearance of the characteristic cornified cells in the vaginal contents. The reaction time is fairly regular. Allen and his co-workers (24) reported that the initial changes in the vaginal smear of the ovariectomized animal could be detected 40-48 hours after injection, but active growth in the vagina and uterus precede these changes. Many workers have since shown that the latent period for the change in the vaginal smear is about two days. The reaction time is not affected by the size of the dose—20 units produce no quicker effect than 2 units (82, 138). Also, the age of the animal does not affect the time, since immature animals show the characteristic changes as soon as adults.

The methods of collecting the vaginal contents vary in detail, and individual workers have their own particular methods. A more serious lack of uniformity in the vaginal smear technique is that while most workers consider that the appearance of full œstrous symptoms, including cornification, is essential for a positive reaction, others, including Laqueur and his collaborators (345) and Lipschütz (372), have used the proœstrous smear as indicating a positive result. Loewe and his co-workers (416) make a cell-type count on the vaginal contents and analyse the results for œstrous periodicity. This method may result in an early proœstrous smear being considered as a positive result, and in any case it is unnecessarily cumbersome. In spite of the variety of intermediate smears which can be obtained, as pointed out by Brouha and Simonnet (96, 97), the vaginal smear technique has a comparatively clear end-point, and the real difficulty in the assay of œstrus-producing extracts is not to determine whether or not œstrus has been produced in any one animal, but to arrive at some generally applicable basis for dealing with the variation found in individual response to a given amount of the hormone. The use of the proœstrous smear as a positive reaction can be supported on the grounds that a small



amount may be required to produce it, and therefore that greater accuracy can be obtained. The proœstrous smear is, however, much more likely to be confused with variations of the smear as found during operative diœstrus than is the clear-cut œstrous smear. For this reason the use of the proœstrous smear only is to be deprecated, especially, as Allen and Doisy remark, in new qualitative work.

Another complication in the use of the vaginal reaction as a test of œstrus-producing activity is the question of whether a dose which will cause œstrous symptoms in the vagina will also produce them in the uterus. In other words, is the threshold value the same for the vagina as for the other accessory organs? Examination of the smear found during induced œstrus in the ovariectomized animal reveals many cases where, though true cornification has occurred, the reaction is hardly as strong as is found during œstrus in the normal animal. Correlated with this it is possible to observe many cases where a definite vaginal reaction has occurred without the slightest trace of an œstrous condition in the uterus. This seems to show fairly definitely that the vaginal reaction has a lower threshold value than has the uterine. Brouha and Simonnet (96) state that the vaginal reaction may be induced without the uterine changes in immature females, and the absence of mating instinct in adults is also attributed to a different threshold requirement of œstrin. In the writer's experience, however, even excessive doses do not result in copulation.

A more serious difficulty has recently become evident in the use of the vaginal smear technique. The cornification of the vagina of the rat, mouse, and guinea-pig was originally thought to be absolutely specific for œstrus, especially since attempts to produce cornification in test animals with various non-specific substances have had negative results. Thus, Robinson and Zondek (538) obtained no result from injection of choline, arginine, guanidine, tyrosine, tyramine, alanine, and asparagine. These authors found, however, that histamine and adrenalin produced hyperplasia of the uterus, but Levin (361) showed that no cornification is produced by the former substance. Frank and his co-workers (230) obtained negative results from olive oil and linseed oil, while yohimbin



(420), and cocaine also produce no effects. Gsell-Busse (252) has recently reported that commercial sodium taurocholate may have an œstrus-producing action. Very recently, however, it has become clear that vaginal cornification may be produced under other conditions than œstrus. Evans (183), for instance, has shown that vitamin A deficiency, which, according to Wolbach and Howe (643), causes cornification of all mucous surfaces in the body, results in cornification of the vaginal mucosa, even in the ovariectomized animal. Thus under certain conditions, cornification of the vagina is not entirely specific for œstrus, and it is evident, therefore, that while the reaction may be an adequate test in the assay of preparations known to contain the œstrous hormone, new qualitative work must be confirmed by the investigation of every possible criterion of œstrus. The uterine reaction, for instance, may be studied in confirmation of the vaginal test, although the difference in threshold value mentioned above complicates this method. The necessity for autopsy at every test is also a disadvantage. The ferret, which has a vaginal reaction at œstrus not connected with cornification, might be used for confirming the vaginal diagnosis on rats and mice in new qualitative work, but the inconvenience of the ferret for laboratory purposes counterbalances the advantage of the external sign of œstrus.

*The immature animal.* The injection of œstrin into the immature animal brings about the appearance of precocious œstrous symptoms, including opening of the vaginal orifice, and where such symptoms may be detected in the intact animal, as in the rat and mouse, this is a fairly convenient test for œstrus-producing activity. It suffers, however, from two disadvantages (*a*) the possibility of spontaneous ovarian activity, or of anterior pituitary effects (see p. 165) and (*b*) the difficulty of using such a test in the standardization of œstrin.

*Other criteria.* An entirely different method of assaying œstrous activity has been put forward by Frank and his co-workers (222), by Seckinger (555) and by Brouha and Simonnet (103), who have suggested that the effect of the œstrous hormone on the spontaneous contractions of the uterus (see p. 203) could be used as a criterion. Such a proceeding, however, has obvious disadvantages. Blotevogel and his co-workers (71-74) have

described a decrease of the chromatophil cells of the cervical ganglion of the mouse after castration. They found that such cells amounted to 2.5% in the normal animal, 1.0% after castration, and 15% during pregnancy. Since the injection of œstrin into the ovariectomized animal raised the percentage, they suggest this as a possible test for the hormone. It is obvious, however, that no technique for the assay of œstrus-producing activity has the ease and reliability of the vaginal smear, and, provided that all anomalous results, such as the extraction of œstrus-producing substances from male urine and plants, are checked by means of other criteria, the vaginal smear technique is probably adequate.

*Ovarian regeneration.* The basis of the assay of ovarian extracts at the present moment is the absence of the ovaries, and it is thus necessary to consider the possibility of the appearance of ovarian tissue in ovariectomized animals. Such tissue may occur in one or both of two ways:

- (a) A small percentage of animals have accessory ovaries which may escape removal at the time of ovariectomy (see p. 12).
- (b) Even after the complete removal of all ovarian tissue at the time of operation, new ovarian tissue may regenerate.

As regards accessory ovaries, it is safe to say that their occurrence is rare (p. 12). In any case, the presence of ovarian tissue, apart from the two normal ovaries, would presumably result in the continuation of the cycle after double ovariectomy, and this would indicate at once, either that the two normal ovaries had not been completely removed, or else that accessory tissue was present. The regeneration of ovarian tissue presents, however, a more difficult problem. This phenomenon was noted by Castle and Phillips (116) in the guinea-pig. These authors reported ten cases of regeneration after ovariectomy of 74 guinea-pigs and 17 rabbits. In three of these animals, litters were produced after ovariectomy. Subsequently the same authors reported eleven regenerations in 141 ovariectomized guinea-pigs. In these cases, however, no criteria of complete initial removal appear to have been available. In view of the

extraordinary hypertrophying power of an ovarian fragment, such criteria are very desirable. Castle and Phillips also state that where ovarian regeneration takes place, the usual ovariectomy effects are not found. Subsequently, Davenport (153), working on mice, reported the results of an extensive series of operations, most of which, however, were only unilateral. His results show that regeneration is much more probable when the ovary alone is removed, than where the capsule and Fallopian tube are also excised. The percentage of regenerations found by Davenport (given below) was remarkably high :—

<i>Ovary only removed</i>								<i>per cent. regeneration</i>
Unilateral	-	-	-	-	-	-	-	67·2
Complete	-	-	-	-	-	-	-	83·3
<i>Ovary, capsule, and tube removed</i>								
Unilateral	-	-	-	-	-	-	-	17·0
Complete	-	-	-	-	-	-	-	16·8

Davenport summarizes his results as follows: ‘Regeneration of the ovary in mice is less apt to occur when capsule, tube, and part of the fat body are removed, than when the ovary alone is removed by cutting its stalk and picking it out of the capsule with least damage to the latter. Regeneration occurs equally in mice that were operated upon when only a month old and in those which were three or four months old. Regeneration may take place within a month or two after operation, but apparently the proportion of regenerated mice increases until six months have passed. Adhesions and minor infections do not inhibit the reformation of the ovary, and such reformation is neither facilitated nor inhibited by pregnancy (in case of unilateral ovariectomy).’

Clinical cases are also on record where pregnancy has occurred in the human after the removal of both ovaries, though such anomalies may be due to the presence of third ovaries. The importance of this possibility of ovarian regeneration in the testing of ovarian extracts has been indicated by Frank and Goldberger (227). The improbability that new ovarian tissue can arise after the removal of the original organs has always fostered suspicion that the apparent regeneration is due to the incomplete removal of the ovaries at the time of



ovariectomy. Haterius (282), denying the possibility of the reformation of ovarian tissue from the coelomic epithelium, considered such an explanation to cover all reported cases.

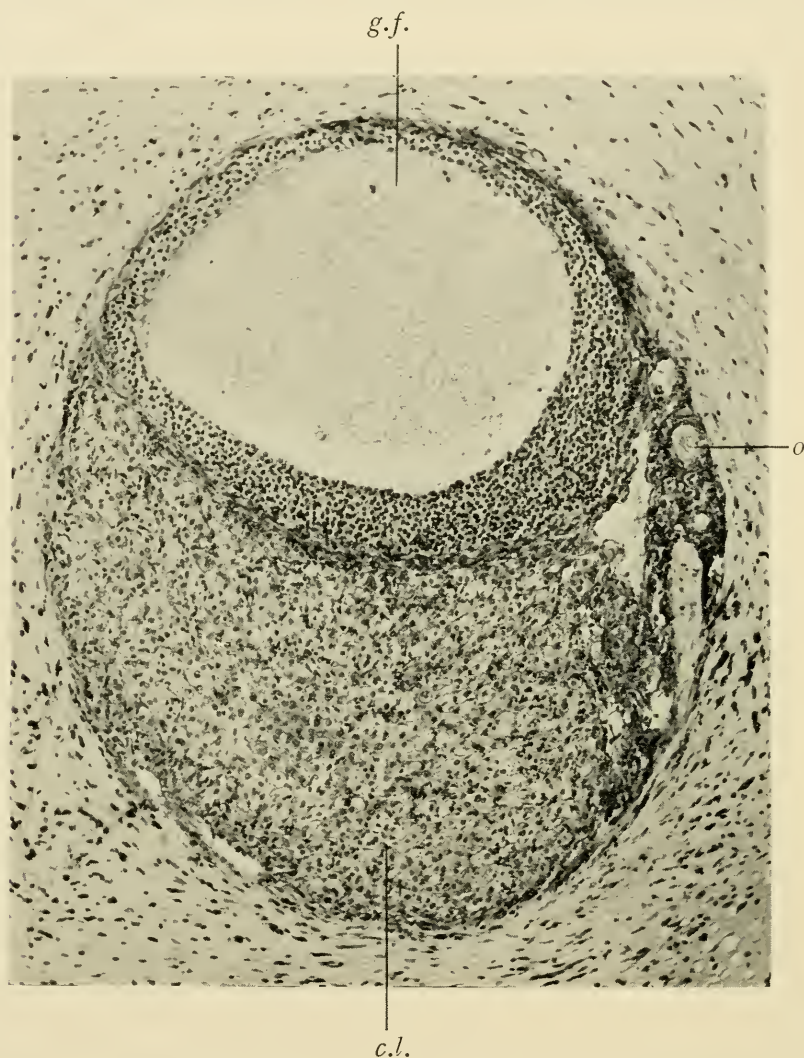


FIG. 43.—OVARIAN TISSUE RE-FORMED AFTER DOUBLE OVARIECTOMY IN THE MOUSE.

The completeness of the original operation was checked by serial sectioning of the ovaries removed.

*c.l.* corpus luteum; *g.f.* Graafian follicle; *o.* oocyte.

Parkes, Fielding, and Brambell (510), however, were able to show quite definitely, by serial histological examination of the organs removed at the time of operation, that ovarian tissue may sometimes reappear after complete excision of the



ovary, capsule, hilum, and Fallopian tube. These authors reported that in 121 double-ovariectomized mice, all of which showed initial cessation of the œstrous cycle, eleven subsequently showed signs of spontaneous œstrus. In eight of these animals the presence of new ovarian tissue was demonstrated histologically, but its exact source was not definitely found. Regeneration was found to occur up to many months after ovariectomy. These authors conclude (*a*) that regeneration can best be detected by the spontaneous reappearance of œstrus, (*b*) that adequate observation for such spontaneous œstrus should be made between each experimental injection, and (*c*) that any anomalous test should be repeated on another animal, and the first one examined for ovarian regeneration.

Hanson and Heys (269) have recently found regeneration in 5% of ovariectomized rats in which the completeness of the original operation was checked by histological examination of the ovaries removed.

#### (*e*) STANDARDIZATION

*The unit.* The unit of œstrin was originally considered to be the amount required to produce full œstrous symptoms in an ovariectomized animal, and a great deal of the initial standardization was carried out on these lines. This technique amounted to giving graduated doses to a series of animals, usually mice or rats, the least amount causing œstrus being considered as the unit. Recently, however, attention has become directed to the extraordinary individual variation in reaction which is found. Two mgms. of a preparation may cause one mouse to come into œstrus, whereas three mgms. may fail to produce this result in another individual. This variation tends to invalidate any such definition of the unit as that given above. Such variation, however, is typical of the pharmacological reaction of animals to many substances, and a method for dealing with the difficulty has now been extended by Coward and Burn (146) to the assay of œstrin. This technique depends on the fact that if a group of animals are injected, the number responding will increase as the amount given increases. Coward and Burn found, for instance,

that 2.5 mgms. of an extract would produce œstrus in about 10% of ovariectomized rats, while 17.5 mgms. would produce œstrus in only a little over 80%. The interpolation of intermediate values gave the usual S-shaped curve of the type found by Trevan (614) to be typical of the response of groups of animals to various drugs and poisons. As a result of this work, Coward and Burn suggest that the unit should be defined as the amount necessary to bring 50% of a group of ovariectomized animals into œstrus. Laqueur (345), however, requires a 75% response for a unit of activity. In practice, this technique can be applied fairly easily, once the standard curve for the strain of animals in question has been worked out. Thus, if a trial injection brings into œstrus 40% of a group, the amount required to bring 50% can be arrived at from the standard curve. Even such methods of dealing with the problem hardly make assay quite satisfactory. The size of group suggested by Coward and Burn is 20 animals, but Dodds and co-workers (5) found that using this comparatively small number different groups may show quite a different percentage response to the injection of a given amount. Further, a group may show a different percentage response at different times, and it seems difficult to correlate this variation with the time after ovariectomy, or with any other variable. It would seem, therefore, that, unless prohibitive numbers of animals are used in each group, the assay of œstrin cannot become exact with the present methods.

*Number of injections.* Working with the old fat-soluble preparations, various authors found that a dose given in a series of small injections produced no greater effect than the same dose given as one injection, and, as a rule, therefore, the fat-soluble extracts were administered as one injection. With the recent water-soluble preparations and with the purer fat extracts, however, it has been found that a given amount will produce much less effect when given as one injection than when given as a series of injections at short intervals. This is presumably due to the rapid absorption, and destruction of the hormone in the blood when administered in a comparatively pure form (5, 438).

*Absorption.* With the cruder fat extracts, the reaction to one

large injection would be spread over several days (505), owing presumably to delay in absorption. The effect of purer fat extracts, on the other hand, was less prolonged, probably owing to more rapid absorption. With the present increase in the purity of extracts this problem becomes of importance. It has been found (438) that (a) maximum activity is obtained when the administration of a dose is spread over 24 to 36 hours, with intervals of not more than two hours between injections, and (b) within limits the reaction is not influenced by the volume of the injections.

*Time of testing after operation.* Following ovariectomy, both the uterus and the vagina begin to undergo atrophy, and in time these organs become entirely degenerate. It seems probable that one result of this degeneration would be an entire lack of sensitivity to ovarian activity, and it is necessary to consider, therefore, how far this degeneration can go without invalidating any tests done on such animals. In other words, it is probable that the longer the injection is postponed after ovariectomy the greater would be the amount of hormone required to produce positive effects. Injection at regular intervals might, on the other hand, prevent the atrophy of the organs and maintain sensitivity. No precise experimental work appears to have been done on this problem. Allen and his collaborators (24) report that the duration of the operative anæstrus has very little effect upon the amount subsequently required to produce œstrus. These authors found no decrease in sensitivity up to fifteen weeks after the operation. Kahnt and Doisy (318), however, 'prime' the animals before carrying out a test.

*Weight of the animal.* Following ovariectomy, the female rodent very soon deposits large amounts of fat and greatly increases in body weight. The influence of this increased weight on the amount of œstrin required for a positive reaction has been studied by Bugbee and Simond (110), whose results are summed up in Table 5 (p. 104).

These results show that as the animal gets older and heavier more œstrin is required for a positive reaction, and the assayed strength of a preparation is less. Correcting for the weight of the animal gives a more constant result, and Bugbee and

Simond, therefore, correct to a standard animal weight of 140 gms. by the formula:

$$U = d \frac{140}{P}$$

where  $U$  is the corrected weight or volume of the unit,  $d$  the uncorrected weight or volume, and  $P$  the weight of the animal in grams. The number of rat units per c.c. or gm. of a preparation is thus  $\frac{P}{d \times 140}$ .

Marrian and Parkes (438), however, failed to find that a correction of this nature was necessary.

TABLE 5.—INFLUENCE OF WEIGHT OF RAT ON  
UNIT OF ŒSTRIN  
(Adapted from Bugbee and Simond).

Age of Animal (months)	Rat units per c.c. of preparation tested.	
	No correction for weight of Animal	Correction for weight of Animal
2-3	8.00	7.15
3-4	5.72	7.50
7-8	5.00	8.30
9-10	5.00	8.35
11-12	3.00	6.39

*Weight of the unit.* Little work appears to have been done on the comparative amounts of œstrin required to produce œstrous symptoms in different species, but from the weight effects discussed in the previous section it would seem that very considerable variation must occur. Allen and Doisy (21) state that two to four times the amount is required for the rat as compared with the mouse, but Coward and Burn (146) found that the doses required were identical for these two animals. Bugbee and Simond (110) confirm Allen and Doisy in finding that the rat unit is two to eight times as great as the mouse unit. As regards larger animals no information appears to be available, but it seems doubtful whether a weight for weight ratio will be



TABLE 6.—WEIGHT OF THE UNIT OF OESTRIN.

Source of Extract	Weight of mouse unit or rat unit (Mgms.)	Authority
<i>Whole ovaries</i>		
Pig	64.0 (r.u.)	Doisy, Ralls, Allen, and Johnston (167)
Cow	6.5 (m.u.)	Parkes and Bellerby (503)
Pig	12.6 (m.u.)	" " "
<i>Liquor folliculi</i>		
Maximum purity	0.13 (r.u.)	Doisy, Ralls, Allen, and Johnston (167)
Minimum purity	19.5 (r.u.)	" " "
Unstated	0.0002 (m.u.)	Lipschütz et al (372)
Cow	12.1-2.61 (m.u.)	Parkes and Bellerby (503)
Pig	21.5-14.2 (m.u.)	" " "
Horse	9.4 (m.u.)	" " "
Pig	0.01-0.02 (r.u.)	Ralls, Jordan, and Doisy (524)
<i>Residual ovarian tissue</i>		
Cow	16.9-9.0 (m.u.)	Parkes and Bellerby (503)
Pig	35.0-10.5 (m.u.)	" " "
Horse	19.6 (m.u.)	" " "
<i>Placenta</i>		
Human	25.0 (r.u.)	Doisy, Ralls, Allen, and Johnston (167)
"	27.7-3.83 (m.u.)	Parkes and Bellerby (506)
Cow	19.2-1.9 (m.u.)	" " "
Sheep	21.7-6.25 (m.u.)	" " "
Unstated	0.02 (m.u.)	Steinach and co-workers (593)
Human	0.1-0.01 (m.u.)	Laqueur and co-workers (356)
"	0.006 (m.u.)	Glimm and Wadehn (246)
"	0.0008 (m.u.)	Laqueur, Hart, and de Jongh (355)
"	0.003 (r.u.)	" " "
"	0.010-0.26 (r.u.)	Allan, Dickens, Dodds, and Howitt (5)

TABLE 6.—WEIGHT OF THE UNIT OF ŒSTRIN.

*(continued).*

Source of Extract	Weight of mouse unit or rat unit (Mgms.)	Authority
<i>Female urine</i>		
Pregnancy	·001 (m.u.)	Zondek (648)
„	·22·06 (r.u.)	Veler and Doisy (621)
„	·0016 (m.u.)	Marrian and Parkes (438)
<i>Plant sources</i>		
Yeast	·1 (m.u.)	Glimm and Wadehn (247)
<i>Unstated</i>		
	10·0 (r.u. or m.u.)	Coward and Burn (146)
	2·25	Frank and Gustavson (229)

found in the amounts required. Pratt and Allen (519) indeed claim to have produced clinical effects with very small doses. The purity of an extract may best be stated in terms of the weight of the unit. The weights given by various authors are collected in Table 6. Such figures, of course, can be only approximate. The variety of techniques used for assay, and unavoidable inaccuracies, make strict comparison impossible.

#### (f) DISTRIBUTION

Extracts were originally made only from animal tissues, but recently the presence of the œstrus-producing hormone has been detected in body fluids. In addition, œstrin or œstrin-like substances have been obtained from various plant sources.

*Distribution in the ovary.* The ovarian follicle was originally used for extraction of the hormone and has long been considered its essential site of origin. Allen and his co-workers (24) by the use of centrifuged and filtered material showed that œstrin is actually present in the liquor folliculi and not merely in the cellular contents which are aspirated at the same time. Zondek and Aschheim (650) claim to have shown by their implantation method (see p. 91) that the hormone is present in the theca

interna cells, especially in atretic follicles, and absent in the follicular granulosa, the ovarian stroma, and the germinal epithelium. The hormone has, however, been shown to occur in residual ovarian tissue, after removal of large follicles and corpora lutea, in much larger amounts than could be accounted for by the remaining follicles (158, 503). Various workers have noted œstrin in ovarian cysts (24, 488). Much controversy has raged around the alleged presence of œstrin in the corpus luteum. Early workers were almost unanimous in supposing that œstrin could be obtained from the corpus luteum. Thus, Iscovesco (313), Seitz, Wintz and Fingerhut (558), Fellner (192), and Herrmann (292) all obtained active extracts from this source. Some of these workers used growth of the rabbit uterus as a test-object, so that the possibility of other than œstrus-producing substances giving the reaction is not excluded. Their illustrations, however, give no suggestion of any reaction except the œstrous condition. Okinschitz (481) failed to obtain positive results from corpus luteum. More recently, positive results have been obtained by Frank and Gustavson (229), Fraenkel and Fonda (218), by Glimm and Wadehn (245), and Zondek and Aschheim (654). Allen and Doisy failed to obtain positive results with corpora lutea from the cow and pig, except in one case of tertiary liquor folliculi, while Johnston and Gould (316) failed with pig corpora lutea. Brouha and Simonnet (93) also obtained negative results from cow corpora lutea. Allen and Doisy did, however, find a slight activity in the hollow human corpora lutea during the early stages of development. The discrepancy in these observations is probably due to the fact that, in the cow particularly, many corpora lutea contain a fluid centre derived from the remains of the liquor folliculi. This fluid might reasonably be supposed to contain œstrin. It is unlikely that the corpus luteum itself, an organ whose development is always associated with the absence of œstrus, would produce the œstrous hormone. Parkes and Bellerby (507) found that in the cow's corpus luteum the fluid enclosed in the centre of hollow specimens contained œstrin, but that the tissue of solid corpora lutea possessed no activity whatever. If, as this work suggests, the œstrin content of the corpus luteum is

restricted to the fluid centre, it seems probable that the corpus luteum tissue does not elaborate the hormone. As regards the ovary, therefore, it may be said that the liquor folliculi of all mammals which have been examined contains this hormone, as does the stromal tissue, but that the corpus luteum contains it incidentally or doubtfully.

*Distribution in other animal tissues.* It has been known for a long time that the placenta contains large amounts of œstrin. Fels (202) failed to find it before the second month of pregnancy, but Allen, Pratt, and Doisy (25) found it in placentae of the third month, and Fellner (191, 192), Herrmann (292), Fraenkel and Fonda (218), Zondek and Brahn (661), Frank and co-workers (229, 231), Allen, Doisy and co-workers (23, 25, 167), Hartmann and Isler (281), Suzuki (606), Zondek and Aschheim (651), Parkes and Bellerby (506), Ceresoli (117), and Dickens, Dodds, and Wright (158) have all found it at later stages. Æstrin has also been found in the foetal membranes (25), amniotic fluid (506, 132, 93, 203), and umbilical cord (25), but extracts of the foetus itself have given uniformly negative results (25, 167, 506).

Various other body tissues have been examined either with a view to ascertaining the distribution of œstrin in the body or with a view to providing control experiments on the extraction of ovaries. Negative results have been obtained from liver (230), brain (230), thymus (230), pancreas (167), pituitary (538), adrenal (654), thyroid (651), spleen (654), and muscle (503).

*Distribution in body fluids.* Since it is obvious that the hormone must pass from its site of origin to its site of activity by way of the circulating blood, one would expect to be able to detect its presence in the blood provided that sufficiently small amounts could be recognized. Also, since its action is cyclic, it might be expected that periodic variation in amount in the blood could be demonstrated. Its presence in the blood of the non-pregnant female has been shown by Loewe (408), Frank and co-workers (225, 227), and Smith (574). Frank and co-workers (223) found œstrin in the blood of the œstrous sow, and in the human in amounts varying with the stage of the cycle. According to these workers, it is present in greatest amount



about the first day of menstruation, after which it rapidly decreases. It is present in menstrual blood in larger amounts than in the circulating blood. Frank also uses the blood œstrin test to determine intersexual conditions. Fels (202) and Trivino (615) found œstrin in the circulation only during pregnancy. Aschheim (36) reports negative results from premenstrual blood, inter-menstrual blood, and blood during labour.

During pregnancy, however, very considerable amounts of œstrin are present in human blood, as shown by Zondek and Aschheim (657), Fels (202), Trivino (615) and Smith (574). Fels describes it as increasing rapidly in the circulating blood after the sixth month of pregnancy, while Aschheim (36) found it only after the fourth month. It is then so plentiful that 2 c.c. of blood serum will give a positive reaction.

The detection of œstrin in blood soon led to an examination of the possibility of its excretion. The presence of œstrin in small amounts has been described in the urine of the non-pregnant female (413), varying according to stages of the menstrual cycle, but during pregnancy relatively enormous amounts have been found by various workers, including Aschheim and Zondek (38), Slotta (570), Zondek (648), and Veler and Doisy (621). Aschheim and Zondek (40) state that it is doubtfully present during the first two months of pregnancy, and as small amounts are found in the urine of the non-pregnant female, this is not essentially a characteristic of pregnancy. They described (38) the concentration in the urine of late pregnancy as 1,000 m.u. per litre. Subsequently, the same authors (40) gave the yield as 4,000-10,000 m.u. per litre (see p. 168). The human female thus seems to excrete about a million mouse units during pregnancy. The comparatively rapid disappearance of œstrin from the urine after parturition has been noted by Aschheim and Zondek (see p. 167) and Veler and Doisy (621). The latter give their results as in Table 7 (p. 110).

*Occurrence in the male.* The presence of œstrin in the testis and also in the male body fluids has been reported by various authors. Fellner (195), Laqueur and de Jongh (357), Brouha and Simonnet (106) and Robinson and Zondek (538) have all

claimed to have secured active extracts from the testis, but Allen and his co-workers (21) failed to obtain this result.

Hirsch (301) and Frank and Goldberger (228) obtained a number of positive results from male blood.

TABLE 7.—ŒSTRIN IN POST-PARTUM URINE.

Time Post-Partum (hrs.)	R.u. per Litre
8.5	640
37	500
48	80
72	22
96	20
144	<5
192	<5
264	<4

Several authors (162, 348, 422, 437) have reported the preparation of œstrin from male urine. Loewe (422) even states that œstrin can be fractionated from the male sex hormone in the urine. It is certain that small amounts (1-3 m.u. per litre) of a substance which will cause cornification of the vagina of the rat and mouse can be obtained from male urine, but in view of recent doubts of the absolute specificity of this test, it is necessary that every possible criterion of œstrus should be used before the substance is definitely identified as œstrin (437).

*Distribution in animals other than mammals.* Little is known about the non-mammalian distribution of this hormone. Fellner (198) claims that it may be detected in the eggs of hens and fish, but Doisy and co-workers (167) were unable to confirm this.

*Occurrence in plants.* Various workers have claimed that an œstrous reaction may be produced by the use of extracts of various plants. Thus, Loewe, Lange, and Spohr (418) say that an extract of willow catkins gives a positive reaction, and that as much as 200 mouse units may be obtained from a kilogram of fresh material. The stigmata of willow blooms contain a small amount (<14 m.u. per kg.), while the stalks and flowers of

TABLE 8.—YIELDS OF OESTRIN FROM VARIOUS SOURCES.

Source	Yield per kg. or litre (mouse units or rat units)	Authority
<i>Whole Ovaries</i>		
Cow	293 m.u.	Parkes and Bellerby (503)
„ (immature)	73-350 m.u.	„ „ „
Pig	219 m.u.	„ „ „
„ (immature)	166-273 m.u.	„ „ „
Pig	120 (r.u.)	Doisy, Ralls, Allen, and Johnston (167)
Sheep (ancestrous)	203 (m.u.)	Parkes and Bellerby (503)
<i>Liquor Folliculi</i>		
Human	433-7000 (r.u.)	Allen, Pratt, and Doisy (25)
Cow	37-788 (m.u.)	Parkes and Bellerby (503)
Horse	113 (m.u.)	„ „ „
Pig	23-75 (m.u.)	„ „ „
„ (average)	878 (r.u.)	Ralls, Jordan, and Doisy (524)
„	600-1600 (m.u.)	Laqueur, Hart, de Jongh, and Wijsenbeek (356)
„	167 (r.u.)	Dickens, Dodds, and Wright (158)
<i>Residual ovarian tissue</i>		
Pig	225 (r.u.)	Dickens, Dodds, and Wright (158)
Cow	150-326 (m.u.)	Parkes and Bellerby (503)
Pig	227-865 (m.u.)	„ „ „
Horse	27 (m.u.)	„ „ „
<i>Corpora lutea</i>		
Human	3700 (r.u.)	Allen, Pratt, and Doisy (25)
Pig	>8 <25 (r.u.)	Allen and Doisy (21)
Cow (unsorted)	16 (m.u.)	Parkes and Bellerby (507)
„ (solid)	no yield	„ „ „
„ (tissue of hollow)	11 (m.u.)	„ „ „
„ (fluid of hollow)	184 (m.u.)	„ „ „

TABLE 8.—YIELDS OF ŒSTRIN FROM VARIOUS SOURCES.  
(continued).

Source	Yield per kg. or litre (mouse units or rat units)	Authority
<i>Placenta</i>		
Human	192-2123 (m.u.)	Parkes and Bellerby (506)
"	400-700 (r.u.)	Doisy, Ralls, Allen, and Johnston (167)
Cow		Parkes and Bellerby (506)
" (maternal)	203-3200 (m.u.)	" " "
" (foetal)	143-782 (m.u.)	" " "
Sheep	183-308 (m.u.)	" " "
Human	1500 (m.u.)	Aschheim (36)
"	52-270 (r.u.)	Allan, Dickens, Dodds, and Howitt (5)
<i>Female blood</i>		
Late pregnancy	1000 (m.u.)	Aschheim (36)
<i>Female Urine</i>		
Pregnancy	1000 (m.u.)	Aschheim and Zondek (38)
"	4-10,000 (m.u.)	" " " (40)
"	470-1240 (r.u.)	Velér and Doisy (621)
<i>Plant Sources</i>		
Willow catkins	200 (m.u.)	Loewe (418)
Press Yeast	30 (m.u.)	Glimm and Wadehn (247)
Brewers' Yeast	50 (m.u.)	" " "

the water lily, though giving a definitely positive reaction, have only about 1 m.u. per kg. According to Glimm and Wadehn (247), and to Dohrn, Poll, and Blotevogel (163) œstrin is present in appreciable quantities in yeast and potatoes. It should be emphasized again, however, that the production of œstrin from such anomalous sources must be confirmed in every possible manner before being fully accepted, especially as the proœstrous smear is considered to indicate a positive result by various workers, including Loewe.



## (g) SITE OF ORIGIN

The coincidence of follicular maturation with the occurrence of œstrus, together with the fact that the initial extractions were made from follicular fluid, led to the supposition that the œstrus-producing hormone was essentially elaborated by the mature follicle. More recently, however, the œstrous hormone has been found in situations where it cannot possibly have been elaborated, so that its occurrence in any particular site is not evidence of its origin there. At the time of the first œstrous period no corpora lutea are present in the ovary, and these structures cannot, therefore, be considered as the essential site of origin. In the same way the placenta, also an abundant source of the hormone, is clearly not the essential site of origin. Further, it has been shown that elimination of the Graafian follicles by exposure to X-rays does not prohibit the formation of the œstrus-producing hormone, and it would seem, therefore, that under certain conditions, if not normally, the stromal tissue of the ovary can elaborate the hormone.

Any hypothesis as to the site of origin of the hormone is necessarily influenced by the view taken as to the exact nature of its function (see Chapter VII).

## (h) PHARMACOLOGICAL PROPERTIES

The pharmacological properties, like the chemical properties, can only be arrived at from negative results; any positive effect may be caused by impurities.

*Effect on circulation.* According to Allen and co-workers (24), Fraenkel (212), Laqueur (346), Doisy, Ralls, and Jordan (166) and Brouha and Simonnet (93), the effect of œstrin on blood pressure is negligible, though crude extracts have a depressor effect. The heart rate is unaltered. Laqueur (346) found no action on the isolated frog heart.

*Respiration.* Laqueur (346) found no effect on the respiration of narcotized cats, and later (357) no effect after injection of 2,000 m.u. into the intact dog.

*Metabolism.* Laqueur, Hart, and de Jongh (346) found no effect on the blood sugar, but a slight increase in the basal

metabolism of ovariectomized females following injection of œstrin. No effect was observed in castrated males. Fraenkel (212) failed to raise the total metabolism of women by similar treatment. Dickens, Dodds, and Wright (158) have reported an antagonistic action of fat-soluble œstrin to the hypoglycaemic effect of insulin.

*Effect on growth and activity.* Bugbee and Simond (112) state that the injection of œstrin causes a decline in weight of normal and gonadectomized animals of both sexes. Brouha and Simonnet (90) and Allen and Doisy (24), however, found no effect on the growth of the immature animal.

Slonaker (566) and Wang, Richter and Guttemacher (630) have shown that the spontaneous activity of rats undergoes cyclic variation in correlation with the œstrous cycle, the maximum activity occurring at œstrus. This increased activity stops after ovariectomy, but can be brought about in the ovariectomized animal by the injection of œstrin (111). Continuous injection leads to continuous increased activity.

## CHAPTER VII

### THE FUNCTION OF ŒSTRIN

#### (a) ACTION ON TEST ANIMALS

IN test animals, the effect of injection of œstrin in adequate amounts is to produce all the extra-ovarian histological and physiological symptoms of the normal œstrous period, except possibly the mating instinct. The uterus of the ovariectomized rabbit undergoes hypertrophy after injection, until a condition similar to that of the normal œstrous uterus is attained. In the immature uterus this involves a very considerable increase in size. Injection of the ovariectomized mouse, rat, and guinea-pig with œstrin leads to growth of the vaginal mucosa, which ends in the keratinization and sloughing off of the surface layers. In the uterus, typical œstrous changes are also produced, including, in the mouse and rat, the characteristic distension of the lumen and attenuation of the uterine wall. The reactions of these animals have been observed by a large number of workers and are beyond dispute. The administration of excessive doses leads to an exaggeration of the œstrous symptoms analogous with that found in nýmphomania. Other animals in which the action of œstrin has been tested after ovariectomy include the opossum and *Macacus rhesus*.

In view of these reactions of test animals there can be no doubt whatever that œstrin is responsible for the extra-ovarian changes typical of proœstrus and œstrus. The actual mechanism of its growth-promoting action in the organs is not known.

#### (b) ACTION ON NORMAL ANIMALS

The injection of œstrin into the normal animal just before œstrus is due, leads to an intensification of the normal changes in the accessory organs without alteration of the ovarian cycle

(but see 436). During the time when œstrus is normally in abeyance (except in the very young animal), the injection of the intact animal with adequate amounts of œstrin results in the appearance of extra-ovarian œstrus. At certain times, however, notably when functional corpora lutea are present in the ovary, considerable dosage is required to bring on this effect. No ovarian changes characteristic of œstrus occur during such an induced period (see p. 119 for non-effect of œstrin on follicular maturation).

*Prenatal period.* Since the administration of large amounts of œstrin during pregnancy leads to reabsorption or abortion, it is not possible to subject the fœtuses to an excessive additional œstrous stimulus, even if it is assumed that œstrin will cross the placenta. Working with amounts insufficient to produce abortion, Courrier (133) claimed to find growth effects on the fœtal uteri after injection of the pregnant guinea-pig. Allen, Francis, and Craig (23), and Parkes and Bellerby (504) were unable to confirm these results on the rat and mouse, while negative results have also been obtained by Loeb (404) on the guinea-pig. Fels (202) claims to have found œstrin in fœtal blood. In view of the large amounts of œstrin in the circulating blood of the pregnant female (see p. 109), the passage of œstrin across the placenta would mean that all fœtuses, including the males, would develop under the influence of this hormone. Courrier's result is therefore improbable. Parkes and Bellerby (504), in fact, found that the uterus of the new-born mouse did not react to œstrin.

*The pre-pubertal animal.* The effect of œstrin injection on the normal pre-pubertal animal has already been indicated in discussing the use of such animals for testing (see p. 93 and also p. 97). In the rat and mouse rapid proliferation of the vaginal epithelium is produced and the vaginal orifice appears. The uterus shows the typical distension, with change of the undeveloped epithelium to a low columnar type. According to Brouha and Simonnet (90) the mammary gland shows no change. Since the same œstrous reaction is found in the ovariectomized immature animal, it is clearly independent of any primary effect upon the existing ovary.

Loeb (405) has produced similar œstrous changes by injection



of œstrin into the immature guinea-pig, while Laqueur and de Jongh (357) have caused growth in the uterus of the immature dog.

*The anœstrous animal.* Asdell and Marshall (45) report the induction of early proœstrous changes in the anœstrous dog by œstrin injection. Courier (134) induced œstrous conditions in the hibernating hedgehog by the same means.

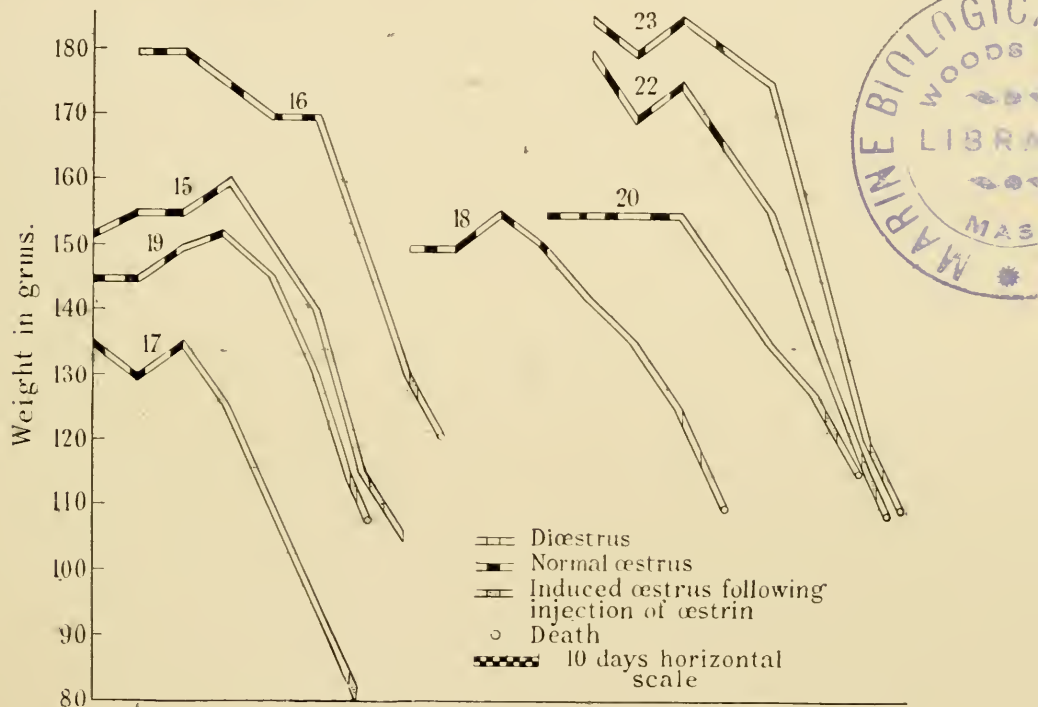


FIG. 44.—DIAGRAM SHOWING THE GROWTH CURVES AND ŒSTROUS CYCLE HISTORIES OF RATS INJECTED WITH ŒSTRIN DURING VITAMIN B DEFICIENCY ANŒSTRUS.

Where anœstrus has been induced in the normal animal by dietary deficiency, it is also possible to cause œstrous changes in the accessory organs by injection of œstrin. Thus Parkes (497) was able to bring about such changes during the anœstrous periods caused by vitamin B deficiency. No ovarian stimulation occurred.

*The diœstrous or pseudo-pregnant animal.* The diœstrous interval of the rat and mouse is too short for accurate experimental work to be done during this phase, but the diœstrous phase in the accessory organs can be almost eliminated by

continued œstrin injection (see p. 126). Both Mahnert and Siegmund (436) and Brouha and Simonnet (91) found that continued injection of the normal animal leads to long periods of cornification separated by short diœstrous intervals. Since the ovarian cycle is disturbed only slightly, if at all, and since persistent cornification is easily produced in the normal immature animal, this result may be due to the cyclic development of the corpus luteum. There can be little doubt that the continuous injection of large doses would lead to persistent cornification in the normal animal.

*The pregnant animal.* Since œstrus is invariably absent during pregnancy, the artificial induction of œstrus during gestation would seem to promise results of interest. Striking effects have actually been obtained. Allen, Francis, and Craig (23) obtained a positive œstrus smear during early pregnancy as the result of injection of œstrin, but Brouha and Simonnet (91) failed to do so in the later stages. Smith (573) showed that pregnancy in the rat could be interrupted in its early stages by the injection of the œstrus-producing hormone, while Parkes and Bellerby (504) found that pregnancy in the mouse could be terminated at all stages by the administration of an adequate dose. The amount required during the later stages was twice as great as in the early stages. Loeb and Kountz (405) failed to interrupt pregnancy in the guinea-pig by similar means, but this was probably due to inadequate dosage. Fraenkel (212) also failed to produce the effect in the rabbit. Zondek and Aschheim (659) produced abortion in the mouse even in the last half of pregnancy by using large doses (10 m.u.), and Fels (205) reports the same result. The facts seem to be well authenticated as regards rats and mice. The effect may be brought about by either or both of two actions. In the early stages of pregnancy the effort of the uterus to assume an œstrous condition may result in the failure of the embryos to become implanted, or, on the other hand, the injection of œstrin, by overriding the action of the persistent corpora lutea, may bring about a state analogous with that produced by removal of the corpora lutea during pregnancy.

Abortion has also been reported by Engle and Mermod (179) as a result of the artificial production of œstrus during pregnancy by the injection of the œstrus-stimulating preparations of the

anterior pituitary body. Zondek and Aschheim (659), however, produced ovulation but not abortion by the same means. The bulk of the evidence suggests most strongly that œstrus and pregnancy are incompatible, and that the artificial production of an œstrous phase during pregnancy will result in its termination.

*The lactating animal.* During lactation, when œstrus is in abeyance in the rat, mouse, and guinea-pig (except for the immediate post-partum period), injection of œstrin will result in the appearance of œstrous symptoms in the accessory organs (23, 505, 565). Parkes and Bellerby (505) came to the following conclusions:

- (a) The amount of œstrin required to produce œstrous changes is roughly proportional to the number of young suckling. It is well known that if only one or two young are suckling, spontaneous œstrus will appear during lactation. With three or four suckling several m.u. of œstrin are required, while with seven suckling not less than 10-12 units will produce œstrus. This is probably a corpus luteum effect (see p. 183).
- (b) The induction of œstrus does not materially affect the efficiency of lactation, but a slight break in the growth curve of the young was found following injection of the mother.

*The senile animal.* Slonaker (568), Steinach, Heinlein, and Wiesner (594) and Laqueur, Hart, and de Jongh (345) report the production of œstrous changes in the senile anœstrous mouse by the injection of œstrin. The last authors obtained the result with 2 m.u. Later, Steinach, Kun, and Hohlweg (596) report the complete rejuvenation of the senile mouse by œstrin, including the recommencement of follicular maturation and ovulation.

*Effect of œstrin on follicular maturation.* Injection of the immature animal has been described by Loeb (405) and Truffi (616) as stimulating the development of the follicles, without, however, causing ovulation. Frank, Kingery, and Gustavson (224) originally claimed to have caused ovulation in the immature rat, but their results were probably due to the use of animals approaching the first œstrous period. All other workers appear to agree that œstrin injection does not cause

ovulation when it would not otherwise occur, either in the immature or the adult animal. Allen and Doisy (19), Brouha and Simonnet (90), and Zondek and Aschheim (652) have all arrived at this conclusion. Parkes and Bellerby (504-5) found that copulation at an œstrous period induced either during pregnancy or lactation never resulted in conception, and thus concluded that ovulation did not occur.

Similar results have been obtained by Slonaker (568), and Steinach, Heinlein, and Wiesner (594) on the senile mouse. After the menopause, when the cycle has stopped, injection of œstrin will bring about cornification of the vagina without the corresponding ovarian changes. Exhaustion of oocytes may have been responsible for the absence of ovulation under these conditions.

According to Mahnert and Siegmund (436) injection of œstrin into the normal adult tends to retard ovulation.

*Clinical results.* Owing to the difficulty of administering adequate doses of the oily extracts to women, clinical research with œstrin has not progressed far. With the new water-soluble preparations it should be possible to make a great advance in this direction. Even so, however, it is far from obvious what part œstrin may be able to play in the correction of reproductive disorders in the human. The premenstrual growth of the uterus is an effect of the corpus luteum, and at least a part of the ensuing menstruation is pseudo-pregnant degeneration (see p. 66). Fraenkel (213-4) in particular has emphasized the improbability that œstrin plays a dominant part in the human cycle. Nevertheless, a variety of positive results have been reported. Wintz (640) and Seitz, Wintz, and Fingerhut (558) record a number of clinical tests of corpus luteum preparations—‘lipamin’ and ‘luteolipoid,’ some of which appeared to give positive results, but it is very difficult to assess this early work. The same applies to the clinical work of Herrmann (292).

Using definitely œstrus-producing preparations, Pratt and Allen (519) claim to have produced enlargement of the human uterus in cases of both primary and operative amenorrhœa. Menstruation was not produced. Zondek (646) obtained more satisfactory results, while Brouha and Simonnet (104) claim to



have successfully treated ovariectomy atrophy, amenorrhœa, sterility, dysmenorrhœa, infantile conditions of the genitalia, and menopause symptoms. Laqueur (357) reports some success in treating amenorrhœa.

It is clear that in considering all such clinical work, due attention must be paid to the difficulty of adequately controlling work on the human, and also to the psychological factor involved. Little can be deduced from the results at present, but it seems probable that œstrin will have some clinical use, especially in expediting labour (see p. 205).

Murphey and his co-workers (471) have reported some trials of the value of œstrin in veterinary practice.

*Action on the male.* Various authors have investigated the effects of œstrin on the male, particularly upon the testis. Adverse effects were reported by Herrmann and Stein (295) who found that the attainment of maturity was delayed in young male rats and rabbits by injections of ovarian lipoids. Heavier doses produced degenerative changes in the testis similar to those caused by X-rays. Fellner (195) confirmed these results, but found that the administration of testis lipoids had the same effect, which was therefore probably not specific. Gould and Doisy (see Allen and Doisy, 21) arrived at a similar conclusion. Bugbee and Simond (112) failed to find any adverse effects on the male. Fels (204) has described an inhibiting action of blood serum of pregnancy on male genitalia, probably due to its œstrin content. Laqueur and co-workers (345) have recently produced degenerative testis changes with a comparatively pure preparation of œstrin, and some 'anti-masculine' action may therefore occur. It is difficult to reconcile this conclusion with the presence of œstrin-like substances in the male urine (see p. 110) and possibly in the testis.

*Action on non-mammals.* Riddle and Tange (535-6), and Loewe, Voss and Paas (419) have investigated the action of œstrin in birds, but no definite results have been obtained.

### (c) LIMITS OF ACTION

Many workers have suggested that œstrin is the one and only ovarian hormone, and that it controls all the changes of the

complete reproductive cycle. In addition, the pre-pubertal development of the accessory organs is usually ascribed to the action of the same hormone. This view supposes that the corpus luteum elaborates the œstrus-producing hormone, at least in the early stages of its life, and that the placenta is also an actual site of origin. The germ 'gestational gland' has been coined by Frank and Gustavson (229) to cover the action of Graafian follicle, corpus luteum, and placenta in successively elaborating the 'female sex hormone.' Allen also supposes that œstrin performs all the ovarian functions. 'The continuous availability of this material from the placenta, present in amounts increasing with placental growth with the advance of gestation, is the most logical explanation of the growth of the uterus and mammary glands, and the absence of menstruation during pregnancy' (12). Allen and his co-workers (24), however, failed to obtain œstrin from the corpora lutea, except in the human, and his view therefore implies that the corpus luteum performs no endocrine action whatever (except a temporary one in the human), and is, in fact, merely a histological ornament. Wadehn (627) also supposes that the changes of pregnancy are due to quantitative variation in œstrin, while Zondek and Aschheim come to much the same conclusion (654).

The whole problem really depends on whether the œstrus-producing hormone is responsible for the changes in the post-œstrous phase of the cycle, or whether these changes are under the control of the corpus luteum. The view that œstrin controls the post-ovulation changes is based largely on two sets of facts: (a) that the hormone causes hypertrophy of the accessory organs, including the mammary glands, and (b) that the hormone is present in very large amounts during pregnancy. It has yet to be definitely shown, however, that the hypertrophy which can be produced by œstrin is greater than that occurring during œstrus, and so far we have no evidence as to the significance of the abundance of the hormone during pregnancy.

Animals such as the rabbit and the ferret, which stay on œstrus indefinitely in the absence of copulation, provide strong evidence that the action of the œstrus-producing hormone relates to œstrus only. During the persistent œstrus in these animals the accessory organs will remain in a static condition for

months. Immediately ovulation takes place and the corpora lutea are formed an entirely new phase of growth is initiated. A large mass of work begun by Fraenkel (208-9) and extended by Ancel and Bouin (30-2), Loeb (400), Marshall (444), and Hammond (264), shows fairly definitely that the corpus luteum itself controls the changes of the post-œstrous phase by some specific internal secretion distinct from that associated with the production of œstrus. This work is dealt with in Chapter X, but one further point may be mentioned here. Functional correlation suggests that the corpus luteum and the œstrus-producing substance are to some extent antagonistic. The injection of the œstrus-producing hormone during pseudo-pregnancy will override this stage of the cycle and result in the reappearance of œstrus. In the same way its injection during pregnancy has been shown to lead to abortion or reabsorption of the foetuses. If the apparent antagonism between the œstrus-producing substance and the corpus luteum is a genuine effect, it is difficult to see how the one hormone can control the whole of the fertile cycle.

The controversy about the limitations of the action of œstrin therefore centres round three problems:

- (a) Is œstrin responsible for the pre-pubertal development of the accessory organs before the first œstrus?
- (b) Is œstrin responsible for the post-ovulation changes in the vagina, uterus, and mammary glands?
- (c) What is the significance of the abundance of œstrin in the placenta and body fluids during pregnancy?

These problems are discussed below.

#### (d) ATTAINMENT OF PUBERTY

It has been pointed out above (p. 20) that the attainment of puberty consists of two phases; the gradual pre-pubertal growth of the accessory organs and the sudden appearance of the first œstrous period. There is clearly no reason to distinguish between the causative mechanism of this first œstrous period and that of any subsequent one. The underlying mechanism must be the same, although it is operating for the first time at

puberty. It has been very clearly demonstrated that injections of œstrin will cause the immature animal to show abruptly all the extra-ovarian symptoms of œstrus (19, 652). To some extent one is justified in calling this a condition of 'precocious puberty.' On account of this reaction of the immature animal, it has been supposed by many authors that œstrin is responsible for the initial pre-pubertal development of the accessory organs. The induction of precocious œstrus is, however, no real simulation of the slow and steady growth which takes place in the pre-pubertal organs, and these experiments only show that the first œstrus is produced, like the subsequent ones, by the action of œstrin.

It is possible, nevertheless, that œstrin is the factor concerned in the development of the accessory organs, and that it is present before puberty in amounts sufficient to produce uterine and vaginal growth, but insufficient to cause œstrous symptoms. This view is supported by Laqueur and co-workers (350) who found that uterine growth may be caused by much smaller amounts than are required to produce œstrus. Thus, .000007 mgm. per day for five days, or about .1 m.u. in all, caused growth of the immature rat uterus. Zondek and Aschheim (652) found that treatment with their water-soluble œstrin for 14 days increased the weight of the immature genitalia of mice from 15-27 mgms. to 75-92 mgms.; hence they conclude that œstrin is the initial growth stimulus. Other evidence is lacking, and Fels (202) was unable to find œstrin in the ovary of the new-born child, in which the uterus is already undergoing development.

There is, moreover, some reason to suppose that a separate ovarian factor (presumably hormonal) is required for the initial development and subsequent maintenance of the accessory organs. Marshall (444) states: '. . . The conclusion to be drawn . . . is that the ovarian hormone which produces œstrus or heat is different from that which is responsible for maintaining the normal uterine nutrition.' Schröder (549) reached a similar conclusion.

There is no doubt that in many animals all the extra-ovarian histological and physiological symptoms of œstrus can be produced in the ovariectomized animal by injection of œstrin, but Asdell and Marshall (45) found that in anœstrous dogs injection brought about the changes characteristic of proœstrus.



without those of complete œstrus. Even in rodents, œstrus induced in the ovariectomized animal is incomplete, in the experience of most workers, in that copulation does not take place. Allen and his collaborators (24) report that seven out of eleven rats copulated at induced œstrus, but Parkes, Fielding and Brambell (510) found that of 92 induced œstrous periods in mated ovariectomized mice only seven were accompanied by copulation. Of these seven, three occurred in mice which were found to have regenerated ovarian tissue. Copulation takes place freely during induced œstrus in ovariectomized mice with regenerated ovarian tissue, and during œstrus induced in the normal animal. In the writer's experience, also, ovariectomized rabbits, receiving heavy injections of œstrin, do not show the tail erection reflex, and will not copulate. Lacasagne and Gricoureff (338), however, state that œstrin will produce copulation in the ovariectomized rabbit. So far as these results go, copulation is clearly not usual at induced œstrus in the ovariectomized mouse, and the missing factor may well be that responsible for the initial development of the accessory organs.

On this view, the ovary probably controls the initial development of the accessory organs by means of some basic internal secretion analogous to that produced by the interstitial tissue of the testis. To this constant endocrine activity, the cyclically active hormones of the œstrous cycle are added at puberty. The irregular occurrence of ovarian interstitial tissue and its late appearance even where present, make it highly improbable that it can elaborate this basic ovarian secretion in a manner analogous with the endocrine activity of the interstitial tissue of the testis. Some such assumption is, however, implied by the use of the term 'puberty gland' by Steinach (590) and others to denote both ovarian and testicular interstitial tissue.

(e) ŒSTRIN AND THE CHANGES OF THE  
POST-OVULATION PHASE

If the changes of the whole cycle are under the control of œstrin, obviously the continuous injection of œstrin ought not to result in the prolongation of œstrous symptoms, but in the

appearance of changes characteristic of the post-ovulative phase. Animals such as the rabbit and the ferret which remain on œstrus indefinitely (in the absence of copulation) without showing any other changes, lead one to suppose that prolonged exertion of the œstrous stimulus does not cause changes characteristic of the luteal phase. This supposition can be confirmed experimentally—continued injection of œstrin results in continued œstrus, probably with exaggerated symptoms, but in nothing more. Analysis of the results produced in the various accessory organs by prolonged administration of œstrin fails to show the production of any changes characteristic of the luteal phase.

*Changes in the vagina.* Cornification of the immature or ovariectomized rodent vagina has been prolonged for nine days by Frank, Kingery and Gustavson (224), thirteen days by Parkes and Bellerby (505), two to three weeks by Zondek and Aschheim (652), fifteen days by Mahnert and Siegmund (436), and thirteen days by Tuisk (619), who concludes 'that we shall always find a prolonged œstrus if in any way the threshold concentration of the follicular hormone is maintained in the blood.' Failure to secure persistent cornification in the normal adult with the doses employed may be due to the cyclic activity of the corpus luteum. The evidence is thus against the view that continued injection of œstrin in the ovariectomized animal will produce anything but prolonged cornification in the vagina, which contrasts definitely with the disappearance of cornified cells and the great infiltration of leucocytes which follow ovulation in the normal animal. Continued action of œstrin is therefore antagonistic to the vaginal changes characteristic of the luteal phase.

*œstrin and the post-œstrous uterus.* The growth of the uterus in the non-pregnant animal may be divided into two phases, namely that occurring at œstrus and that occurring after ovulation, presumably as a preparation for the reception of a fertilized ovum. In most animals these two phases are quite distinct and the changes characteristic of œstrus pass off before the onset of those correlated with the luteal phase of the cycle. It is known quite definitely that the œstrous changes in the uterus are brought about by the œstrus-producing hormone, and

accordingly it seems highly improbable that a further set of different changes can be produced by the same agency. In the normal mouse and rat the injection of œstrin after copulation leads to the return of œstrous symptoms, and to the failure of the embryos to become implanted. There is sufficient evidence to state, therefore, that in the rat and mouse the œstrus-producing hormone is not concerned with the production of post-œstrous changes; the experimental prolongation of its activity is directly antagonistic to the changes of the luteal phase. In the guinea-pig and the cow, as shown respectively by Loeb and by Hammond, the antagonism between the follicular phase and the post-œstrous phase has been well demonstrated. These authors showed that removal of the corpora lutea after ovulation led to a much earlier return of œstrus, and in these animals, therefore, the action of the corpus luteum is clearly directly opposed to that of the œstrus-producing substance. Loeb (405), too, has shown that the pre-decidual changes in the guinea-pig uterus are inhibited by the injection of œstrin, and further, that such treatment inhibits the uterine sensitivity to mechanical irritation necessary for the formation of deciduomata. In the rabbit uterus, the prolonged period of œstrus leads to none of the changes which are characteristic of pseudo-pregnancy. Many workers have figured the rabbit uterus after heavy or prolonged dosage with œstrin, and in no case has any appearance of pseudo-pregnancy been produced. Thus Fellner's early figures (192) and a very recent illustration by Laqueur (357) both show the typical œstrous condition, which, as pointed out before (p. 54), is so obviously distinguishable from that of pseudo-pregnancy. The same condition is shown in Courrier's (145) illustrations. Long and Evans (425) have found that the onset of the next œstrus is fatal to the existence of deciduomata, which are known to be directly under the influence of the corpus luteum. Ovulation, therefore, does not merely transfer the elaboration of the œstrus-producing hormone from the follicle to the corpus luteum.

The most obvious post-ovulation changes in the uterus are of course found in Primates, and evidence is now beginning to accumulate as to the effect of various experimental procedures upon this pseudo-pregnant development. Allen (12) has



described remarkable experiments on *Macacus rhesus*. His experimental results may be summarized as follows:

- (1) Ovariectomy before the next menstruation is due leads to its premature appearance. The same effect is produced by the experimental rupture of the large follicles in the ovary.
- (2) The injection of the follicular hormone into the ovariectomized female results in the rapid appearance of the local colouration found at certain stages of the cycle in the female animal.
- (3) When the ovariectomized female has been regularly injected for some considerable period, the cessation of injection is followed by the appearance of menstruation.

The protocols, however, suggest that this last reaction is less regular and less intense than is found in the normal female. Allen explains these results by the assumption that the follicular hormone is responsible for the pre-menstrual congestion of the uterus, and that its secretion is carried on after ovulation by the corpus luteum, and, in the event of pregnancy, by the placenta. These assumptions would explain why menstruation in the normal animal does not appear until some time after ovulation, and why it disappears entirely during pregnancy. If the production of the follicular hormone is stopped prematurely, owing to (a) atrophy of the corpus luteum and the failure of placental tissue to appear, (b) experimental damage to large follicles, or double ovariectomy, (c) the cessation of injection in the ovariectomized animal, uterine retrogression resulting in menstruation sets in. Allen's explanation of his experimental results assumes a continued activity of œstrin from the beginning of the follicular phase to the end of pregnancy.

The whole problem, however, is connected with the question of the interpretation of the menstrual cycle of the Primates. This has been considered in Chapter V, where it was concluded that the phenomenon of menstruation is partly pseudo-pregnant degeneration, and partly a proœstrous occurrence. It is probable, therefore, that the menstruation produced by Allen in the injected monkeys was the proœstrous phenomenon, and it seems possible to interpret all his experimental results on this



basis. Allen was inducing that type of menstruation which is found in non-ovulating monkeys and humans, and which has been shown by Corner (126) to be entirely independent of the true pre-menstrual congestion of the uterus.

*Œstrin and the mammary gland.* Some evidence has been brought forward in support of the view that prolonged injection of Œstrin will cause complete development of the mammary glands. The development of the mammary gland in castrated male guinea-pigs feminized by an ovarian graft (see p. 78) is sometimes interpreted as being due to the action of Œstrin derived from the graft. The condition produced in the castrated male has been likened by Lipschütz (371) to prolonged Œstrus, but, even if no organized corpora lutea occur in the graft, lutein cells are undoubtedly formed by the atretic follicles of the graft (see p. 76), and it is impossible, therefore, to exclude the possibility of luteal activity.

Mammary growth has occasionally been reported as accompanying abnormal persistence of Œstrus in polyŒstrous animals. Thus Courrier (136) has described a guinea-pig in which nymphomania, accompanied by cystic ovaries and prolonged cornification of the vagina, was associated with development of the mammary gland. There is no evidence, however, that the development was equal to that of pregnancy. Probably an exaggeration of the Œstrous development of the glands, which is considerable in the guinea-pig, had occurred.

Very early in the course of work on ovarian extracts it was reported that their injection would produce hypertrophy of the mammary tissue in the ovariectomized or immature rabbit. Since there can be little doubt that all the early extracts of ovary contained only the Œstrus-producing substance as the active principle, this finding seems to suggest some connection between the Œstrus-producing hormone and the mammary gland. Herrmann (291) and Fellner (192) originally reported the effect in the ovariectomized female and castrated male rabbit, and quite recently Vintemberger (624) has confirmed this result. Aschner (41), Frank and Rosenbloom (231), and Loewe (407) have all reported similar effects in various animals. According to Hartman, Dupré, and Allen (279) the reaction of the mammary tissue to Œstrin in the

opossum is very obvious and can actually be detected by palpation of the intact animal. Allen (12) also has described hypertrophy of the mammae in *Macacus rhesus* after the injection of œstrin, while Loeb (405) has dealt with the same effect in the guinea-pig. Laqueur and co-workers (351) have recently, by injecting œstrin, produced hypertrophy of the mammae in normal and ovariectomized female, and normal and castrated male guinea-pigs, in young male dogs, and in castrated monkeys. They consider œstrin to be the normal stimulus for the entire development of the mammary gland. Haterius (283) obtained hypertrophy of the mammary tissue of male guinea-pigs by the same treatment. Similar results on the ovariectomized guinea-pig have been reported by Steinach and co-workers (593).

Superficially, all this would appear to be evidence against the great mass of observational and experimental data which seems to show that the corpus luteum is responsible for the development of the mammary gland. Actually, however, it is highly probable that no such interpretation can be placed upon these results. In the first place, few workers claim to have produced sufficient development in the mammary gland to lead to the secretion of milk. Fellner (192) specifically states that the growth was not enough to allow of milk secretion. It seems most probable that the degree of development of the mammary gland which can be produced by the injection of œstrin is only comparable to that which has been shown to occur normally at œstrus in many species and to be quite independent of the main development during pregnancy, which is under the control of the corpus luteum. In Chapter III it was pointed out that a cycle in the mammary gland in the unmated animal may be just as typical of the œstrous cycle as is the cycle in the uterus and the vagina. Thus, in the guinea-pig Loeb and Hesselberg (402) have shown that proliferation in the mammary gland occurs at œstrus, while Myers (472) has described cyclic mammary changes in the non-pregnant rat. Marshall (444) describes hyperplasia of the mammary gland in the mare at œstrus. Hartman (271) in discussing the œstrus cycle in the opossum also emphasizes the fact that the amount of growth in the mammary gland at the time of œstrus, although consider-

able, is negligible compared with that found during pregnancy or pseudo-pregnancy. In this animal, however, the growth from proœstrus to the end of pseudo-pregnancy does appear to be continuous. Hartman's results show that injection of follicular extract into the ovariectomized opossum produces growth only equal to that of œstrus. In the rabbit the distinction between the œstrous proliferation of the mammary gland and that occurring under the influence of the corpus luteum is most obvious. Ancel and Bouin (34) and Vintemberger (624) distinguish very definitely between the slight mammary proliferation characteristic of œstrus, which can be produced by the injection of œstrin, and the extensive hypertrophy which is characteristic of pregnancy and pseudo-pregnancy. The latter phase of growth does not occur during even the most prolonged œstrus and cannot be caused by the injection of the œstrus-producing hormone. Fellner's (192) extensive illustrations of the effects of his ovarian extracts on the mammary gland make it perfectly obvious that in no case was greater development induced than is normally found at œstrus.

The comparative influence of œstrin on the mammae of the rat and guinea-pig is also instructive. In the former animal the growth produced by œstrin is negligible (565) while in the latter it is considerable (230). This comparison corresponds exactly with the conditions in the normal animals at œstrus.

The evidence that œstrin can cause complete development of the mammary gland is therefore quite inconclusive. Probably, at most, an exaggeration of the proliferation characteristic of œstrus can be produced.

#### (f) SIGNIFICANCE OF DISTRIBUTION

The occurrence of œstrin-like substances in the male cannot be discussed until their identity with œstrin is demonstrated. As regards the non-pregnant female, the distribution of œstrin presents few problems. Its presence in the follicles and stromal tissue of the ovary, its very doubtful presence in the corpus luteum, and the small and varying quantities in the blood and urine, are quite in accordance with expectation.

*Significance of occurrence in body fluids during pregnancy.*

The most difficult problem in connection with the distribution of œstrin is to explain its abundance in the body fluids during pregnancy, *i.e.* during the luteal phase, and to explain why this abundance does not result in the appearance of œstrus and abortion. *A priori* the excess of œstrin during pregnancy suggests strongly that it is responsible for the changes of the luteal phase. Two explanations more in keeping with the mass of evidence discussed above may be put forward:

- (a) In spite of the large amounts of œstrin in the body fluids, the corpus luteum may still be dominant.
- (b) There is some evidence (see p. 188) that the preliminary action of œstrin is necessary for the effective action of the luteal hormone. In this case the presence of œstrin during pregnancy may be a necessary complement to the action of the corpus luteum.

In either case the idea of a balance between œstrin and the corpus luteum (see p. 182) is a necessary assumption. The refinement of methods for obtaining the luteal hormone from body fluids should enable this point to be decided. The source of the large excess of œstrin during pregnancy has usually been put down to its elaboration by the placenta, but Fellner (194) considers it to be due at least in part to the activity of the ovarian interstitial tissue.

*Significance of occurrence in placenta.* The abundance of œstrin in the placenta has led many workers, notably Allen (12) and Aschheim (36) to conclude that elaboration of the hormone is carried on in this organ. It is, however, almost as difficult to assume that œstrin is elaborated by the placenta as to assume the opposite. The only evidence in favour of its elaboration by the placenta is its abundance in the organ, and this is far from conclusive. On the other hand, various workers have shown that the injection of œstrin during pregnancy leads to reabsorption or abortion, and its secretion by the placenta would thus be a definite anomaly. Weight for weight, the placenta contains as much œstrin as the ovaries, and since the weight of the placenta may be anything up to 500 times as much as the ovaries, it contains about 500 times as much œstrin. If the placenta elaborates œstrin, therefore, and at the same rate as the ovaries,



it would produce 500 times as much as the ovaries; yet no symptoms of œstrus occur during pregnancy, and the injection of œstrin during pregnancy leads to abortion. If, on the other hand, the presence of œstrin in the placenta in such large amounts is not due to its elaboration there, it is necessary to have some working hypothesis as to the reason for its occurrence. It has been suggested (506) that the placenta absorbs œstrin from the maternal circulation in order to protect the male fœtuses from its action. As Lillie (363) has pointed out, the male fœtus must be protected in some manner from the sex hormones of the mother, and in view of the reported 'anti-masculine' action of œstrin it is not improbable that some such mechanism does exist.

## CHAPTER VIII

### THE PERIODICITY OF ŒSTRUS

#### (a) RÔLE OF THE CYCLIC STRUCTURES OF THE OVARY

THE intimate correlation between the cyclic changes in the ovary and those in the accessory organs naturally led to the conclusion that the periodic development of the cyclic structures of the ovary was responsible for the cyclic changes in the accessory organs. At first considerable attention was given to the corpora lutea as the main regulators of œstrous periodicity; it was held that their development after each ovulation inhibited a further appearance of œstrus during their functional lifetime. This view was supported by the various experiments in which the removal of the corpora lutea was found to expedite the appearance of the following œstrous period. There are many reasons, however, for concluding that œstrus is not necessarily preceded by the atrophy of corpora lutea. (a) The appearance of œstrus at puberty or after anœstrus occurs when no corpora lutea at all are present in the ovary. (b) In many animals the atrophy of the corpus luteum at the end of pregnancy is not followed immediately by œstrus (rodents, of course, are an exception to this). It may be concluded, therefore, that while the corpora lutea, when caused to persist, undoubtedly have the effect of delaying the next œstrous period, they do not regulate the essential periodicity of œstrus. It follows that if any cyclic structure of the ovary has this function, it must be the mature Graafian follicle, and many observations tend to support this view.

#### (b) RELATION BETWEEN THE GRAAFIAN FOLLICLE AND THE PRODUCTION OF ŒSTRUS

*Functional correlation.* In the normal animal a regular connection is found between the maturation of Graafian

follicles and the appearance of œstrous symptoms in the accessory organs. This correlation is as follows:

- (a) Œstrus and ovulation are synchronized throughout the entire reproductive life of the animal. The first œstrus appears with the first ovulation at puberty and the last œstrus is synchronized with the last ovulation at the menopause. The bat, as pointed out by Courier (131), is an exception to this rule. In this animal œstrus occurs in the autumn and ovulation in the spring.
- (b) Where the breeding season is limited, the beginning and end of the ovarian cycle are correlated with the beginning and end of uterine activity.
- (c) In the ferret and rabbit, where ovulation only occurs after copulation, mature follicles and the œstrous condition persist together indefinitely in the absence of coitus.
- (d) During the luteal phase of the cycle, particularly during pseudo-pregnancy and pregnancy, when no follicles normally mature, no symptoms of œstrus occur.
- (e) A condition of persistent œstrus in animals which normally ovulate spontaneously is sometimes found in conjunction with persistent cystic follicles in the ovary.

Since no uterine effect on the maturation of the follicles has been demonstrated, this functional correlation throughout the whole lifetime of the animal could most easily be explained on the grounds that the œstrous cycle is regulated by the periodic maturation of Graafian follicles.

The tendency to emphasize the importance of the Graafian follicle in the production of œstrus was accentuated when the liquor folliculi was found to contain large amounts of the œstrus-producing substance. Allen (11) claims to have shown that the amount of the œstrus-producing substance which can be obtained from liquor folliculi varies according to the stage of maturation of the follicle. In this connection he remarks (8) 'Its presence and absence, due to the periodic development of successive sets of follicles, is sufficient to explain the mechanism of œstrous phenomena.' This author and his co-workers actually maintain that the Graafian follicle elaborates the œstrus-produc-

ing hormone under the influence of the ovum itself. They state (24) 'From a functional analysis of the follicle through its various stages of growth, it seems probable that the production of this hormone is referable ultimately to the metabolism of the ovum itself as the dynamic centre of the follicle,' while Hartman (271) in discussing the production of œstrus in the opossum says, 'What element of the ovary constitutes the source of stimuli that lead to proœstrus and to œstrus? The opossum affords an unequivocal answer which is in full accord with the clear and succinct statements by both Allen and Robinson, in which they make out a case for the Graafian follicles. Their reasons I consider conclusive.' Robinson's (537) work on the ferret led him to conclude that 'the phenomena of proœstrus and œstrus only appear when a group of follicles has attained a stage of development which may be called pre-inseminal maturity, and the phenomena are due to some secretion produced by the follicles in that phase of their development.' In a recent paper Zondek and Aschheim (650) conclude that the œstrus-producing hormone is elaborated by the theca interna of the follicle. These workers implanted into ovariectomized mice various portions of the human ovary; only the theca interna implants caused a positive reaction. Even very recently Hammond and Marshall (267) have stated in connection with the vaginal œstrous changes in the ferret, 'This outward sign of the production of the œstrous hormone we consider is due to the presence of ripe follicles in the ovaries since it is absent during anœstrus, when only small follicles are present.' All these statements, however, are made on the evidence of functional correlation, and the whole hypothesis that the mature follicle is responsible for the production of œstrus has resulted from the elimination of other probabilities rather than from experimental work.

*Time relation of follicular maturation and operation of the œstrus-producing stimulus.* Evidence that the maturing Graafian follicle is not the causative factor in the production of œstrus is forthcoming from the fact that the real maturation of the follicle only begins after the œstrus-producing stimulus has become active. It has been known for some time (425) that double ovariectomy may be followed shortly afterwards by the



appearance of œstrus, although no subsequent recurrence is found unless ovarian regeneration takes place. A similar observation was made by Coward and Burn (146). The significance of this discovery was not, however, appreciated immediately. Brambell and Parkes (82) found that œstrus in the mouse may occur up to 36-48 hours after double ovariectomy; the only possible explanation of this phenomenon is that the œstrus-producing stimulus becomes operative at about 48 hours before its effect can be discerned by examination of the vaginal smear. Histological examination of the ovaries removed from mice coming into œstrus within the two days following the operation revealed the fact that the real maturation growth of the follicle had not taken place. The average volume of Graafian follicles not due to ovulate at the next œstrous period is about three million  $\mu^3$ . This same size is maintained until about halfway through the diœstrus preceding ovulation. When the œstrus-producing stimulus becomes operative the follicles about to ovulate have increased in size to an average of  $3\frac{1}{2}$  million  $\mu^3$ . During the two days preceding ovulation, enormous follicular growth takes place, so that a follicle at the time of ovulation has a volume of between eight and nine million  $\mu^3$ . These facts show adequately that in the mouse, at any rate, the maturation of the follicle does not occur until after operation of the œstrus-producing stimulus, and cannot, therefore, be responsible for this stimulus.

(c) OCCURRENCE OF ŒSTRUS AFTER FOLLICULAR  
ABLATION

The real test of whether or not the maturation of the Graafian follicle is the causative factor of œstrus is the effect of total ablation of the follicles. The difficulty of completely destroying the follicular system of the ovary has delayed such experimental work. Marshall and Runciman (454) failed to inhibit the onset of œstrus by rupturing the maturing follicles of the dog, and hence considered that the presence of mature follicles was not essential for the production of œstrus. Later, however, Marshall and Wood (455) were unable to confirm these results. Various doubtful points may be raised with regard to these

experiments. If the initial growth of the uterus of the dog had begun when the follicles were ruptured, the removal of the follicles might have led to the immediate onset of proœstrous degeneration. The inhibitory effects produced in the later experiments might be due to the severity of the operation or to the formation of luteal tissue by the ruptured follicles (see p. 184). There is thus no direct experimental evidence that the mature Graafian follicle is the essential factor in the production of œstrus.

Evidence is now available which shows quite definitely that the maturation of Graafian follicles is not necessary for the occurrence of œstrous symptoms in the accessory organs. Blair Bell (56) long ago reported experiments where the grafting of rabbit ovaries from which the cortical areas had been removed resulted in the appearance of œstrus in the host. Since the cortical areas would contain the majority, if not all, of the Graafian follicles, these experiments provided a hint that the follicles were not essential for the appearance of œstrus. The really critical experiment, namely, the investigation of the results of entire obliteration of the follicular system of the ovary, has only recently been attempted. Possible means whereby the ovarian follicles can be eliminated are few, and exposure to X-rays is by far the most certain technique. The action of X-rays on the ovary has been studied to some extent physiologically, and in detail histologically, for many years, and it was soon found that the Graafian follicles and, indirectly, the corpora lutea could be eliminated. Their elimination has been shown to have no effect in inhibiting the development of the accessory organs in the immature animal, or in causing atrophy of the accessory organs in the adult. The early workers on the effects of X-ray sterilization appear to have made no observations on its effects on the occurrence of the œstrous cycle. Recently, however, this problem has been investigated in detail (492-5), and it is possible to state quite definitely that the entire elimination of the whole follicular system neither inhibits œstrus, nor interferes with its normal periodicity.

*Histological effects of exposure to X-rays.* Early work on the histological effects of exposure of the ovary to X-rays was carried out by Halberstadter (261) and by Fellner (190) on the

rabbit. Bergonié and his co-workers (61-3) showed that while the Graafian follicles were caused to undergo complete atrophy, existing corpora lutea were not affected. Bouin, Ancel, and Villemin (78) found that the interstitial tissue remained intact, and according to Steinach and Holznecht (595) the interstitial



FIG. 45.—OVARY OF MOUSE STERILIZED BY EXPOSURE TO X-RAYS WHEN THREE WEEKS OLD.

The ovary is largely composed of new tissue derived from the germinal epithelium.

*f.r.* follicular remains; *n.t.* new tissue.

tissue is actually augmented from the degenerated follicles. Increase in the interstitial tissue was likewise found by Hüssy and Wallart (309) in the human ovary after irradiation. The effects of X-rays on the ovary have also been described in detail by Reifferscheid (529-531).

In the mouse the changes following X-rays have been described in detail by Brambell, Fielding, and Parkes (83-6). In the young animal irradiated before puberty the Graafian follicles undergo complete atrophy and are entirely reabsorbed. This atrophy, which includes both the ovum and the membrana granulosa, eventually involves the theca interna in follicles where it is differentiated. Finally, the degenerated follicles are



represented merely by small cavities and remnants of zona pellucida. In a few cases, larger follicles may become filled with blood and form cysts, or the cells of the theca interna and the membrana granulosa may grow and invade the antrum to form a corpus luteum atreticum. These corpora lutea atretica appear



FIG. 46.—OVARY OF MOUSE STERILIZED WHEN THREE WEEKS OLD, SHOWING CAVITIES WITH REMAINS OF OVA.

*b.v.* blood vessel; *f.c.* follicular cavity.

to persist indefinitely, although no physiological function can be attributed to them. Concurrently with these changes in the follicles, the old interfollicular tissue atrophies and proliferation takes place from the germinal epithelium. This proliferation, forming a parenchymatous tissue, constitutes almost the whole



of the usual type of ovary resulting from sterilization before puberty. Subsequently, a second proliferation from the germinal epithelium may take place in the form of cords resembling small anovular follicles or embryonic testis tubules. The weight of evidence favours the view that they are of the former nature. In certain abnormal mice the first proliferation from the germinal epithelium becomes extremely luteal-like, and, since this tissue forms almost the whole of the sterilized ovary, the

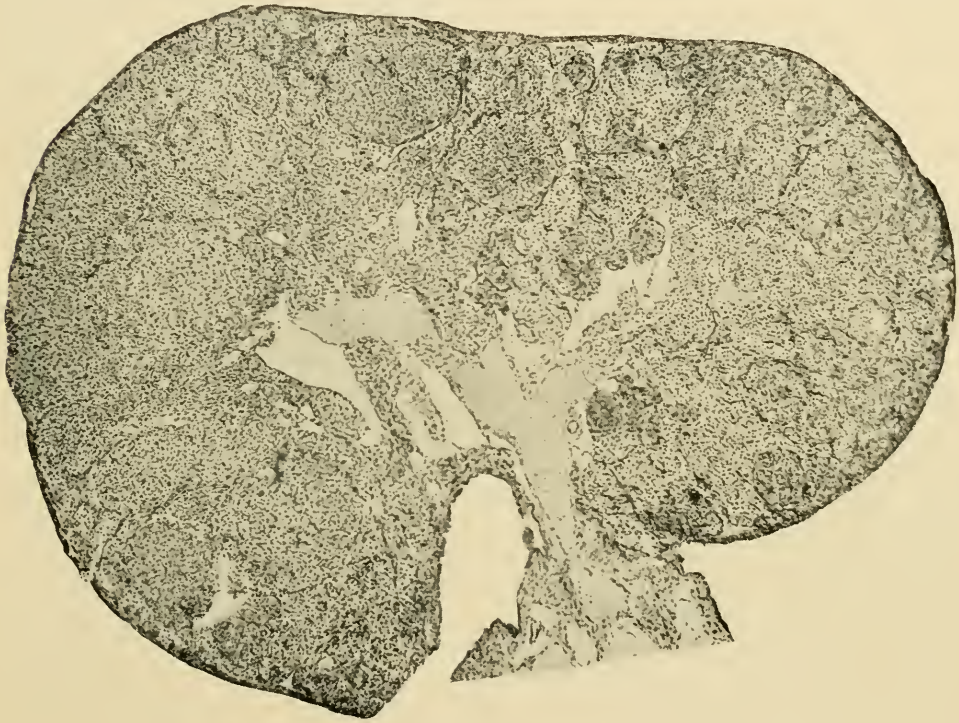


FIG. 47.—OVARY OF MOUSE STERILIZED BY EXPOSURE TO X-RAYS WHEN ADULT.

The ovary is largely composed of tissue derived from the old follicles.

appearance of a single large corpus luteum is produced. This abnormal type of sterilized ovary results more commonly from sterilization immediately after birth than at three weeks old. Animals with the abnormal type of sterilized ovary show physiological abnormalities, including inhibition of œstrus.

Sterilization of the adult mouse leads roughly to the same kind of histological change, but the details are somewhat different. In the irradiated adult, the elements already forming the ovarian cortex at the time of irradiation persist in a changed

form and constitute the bulk of the sterilized ovary. Immediately after irradiation of the adult, the small follicles become atretic without, however, undergoing complete degeneration; the larger follicles (with the dosage used) survive and may eventually ovulate. Thus mice may become pregnant up to

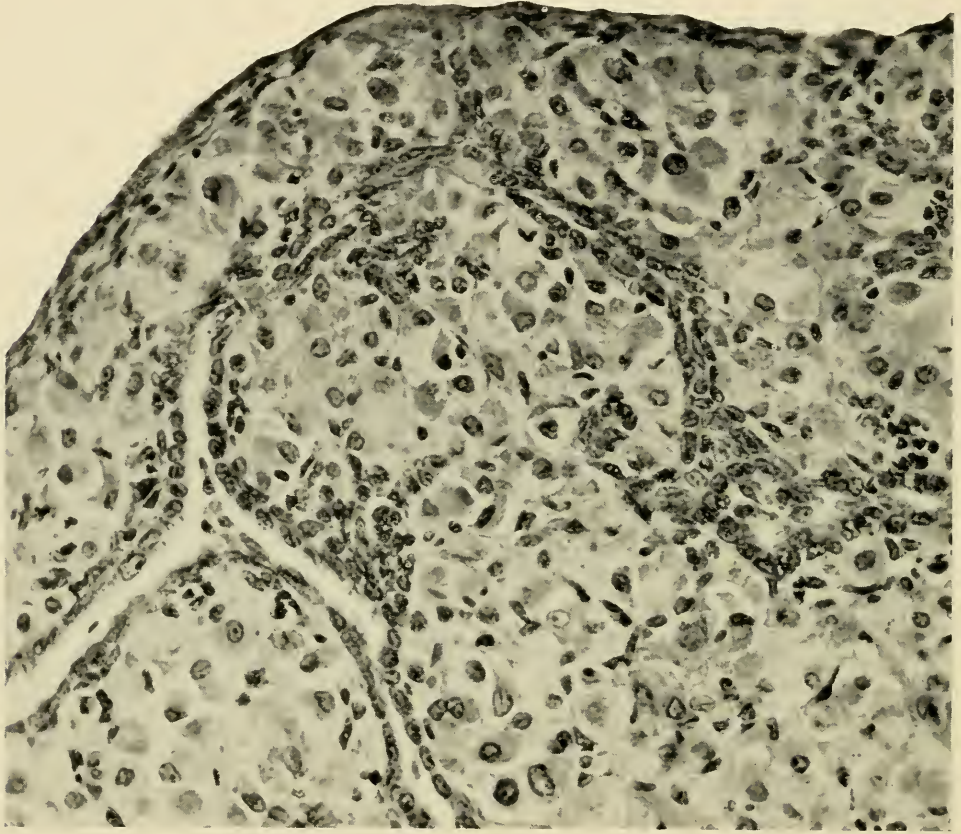


FIG. 48.—OVARY OF MOUSE STERILIZED AT BIRTH.

This shows an abnormal condition of luteinization found in a small percentage of sterilized ovaries.

some ten days after the application of a dose of X-rays sufficient to result in complete sterility later. The remaining tissues of the smaller follicles become entirely disorganized, and the ovary ultimately consists of a more or less uniform tissue derived from granulosa cells. Small anovular follicles are also present in the ovaries of the irradiated adult. They are formed, however, not by proliferation from the germinal epithelium, but by the degeneration of the ovum and the growth of the mem-



brana granulosa cells of primordial follicles. The adult ovary does not show proliferations from the germinal epithelium after sterilization. Such an ovary differs, therefore, from that of the sterilized immature animal in consisting of tissue of follicular derivation. No signs of periodic change have been observed in this tissue once a stable condition has been reached; the histological periodicity appears to be destroyed as completely in the adult as in the immature animal. The abnormal luteal type of

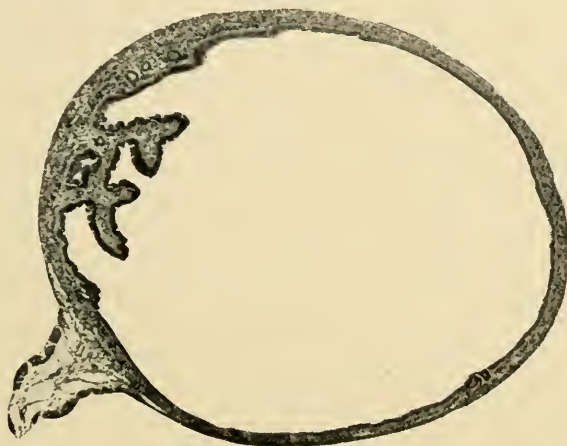


FIG. 49.—UTERUS OF MOUSE STERILIZED AT WEANING TIME BY EXPOSURE TO X-RAYS, SHOWING TYPICAL ŒSTROUS CONDITION (cf. fig. 22).

ovary is found only rarely in the sterilized adult. The effects of exposure to X-rays in adequate dosage may therefore be summarized as follows:

- (a) Destruction, and in the immature animal complete reabsorption, of the Graafian follicle, and indirect elimination of the corpora lutea.
- (b) Complete elimination of cyclic histological changes.

*Effect of X-ray sterilization on the œstrous cycle.* Mice in which the entire follicular system has been destroyed by exposure to X-rays, show the typical œstrous changes in the accessory organs at the same periodicity as the normal female. Sterilization (492-5) has been described at three ages—(a) just before or at the time of birth, (b) at weaning, (c) at maturity. In mice irradiated at weaning time, puberty occurs at the normal date, namely, 6-7

weeks of age, and the subsequent periodicity of œstrus, though slightly erratic in certain instances, is reasonably normal. In mice sterilized at an earlier age, the œstrous cycle tends to be more abnormal. This is due to the fact that abnormal histological effects, such as the luteinization of the cells derived from the germinal epithelium, are more often found. In such animals the cycle is either absent or ceases after a transitory appearance. In most sterilized adults the cycle in the accessory organs persists quite unchanged. Table 9 shows the length of œstrus and diœstrus in twenty mice before and after irradiation.

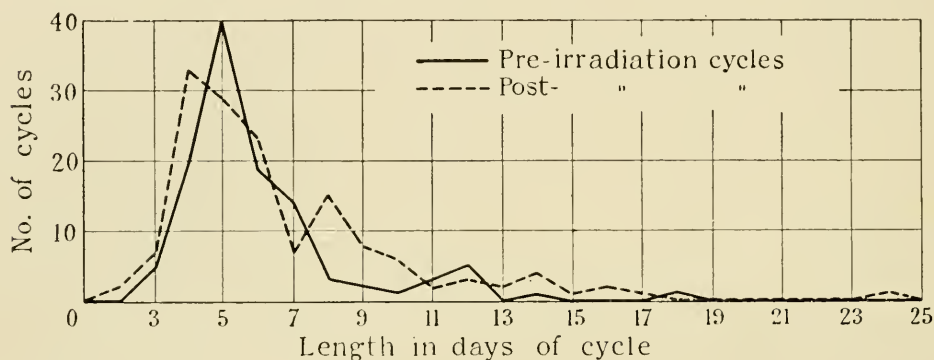


FIG. 50.—FREQUENCY POLYGONS FOR LENGTH OF ŒSTROUS CYCLE BEFORE AND AFTER X-RAY STERILIZATION.

No significant change is observable.

In all these animals histological examination of the ovaries showed that no Graafian follicles whatever were present. The component parts of the cycle, namely diœstrus, proœstrus, metœstrus, and œstrus, were all found to be normal. The only unusual feature of the post-irradiation cycle is its slightly greater variability in length; it is quite evident that this in no way detracts from the general conclusion that the complete destruction of the follicular system of the ovaries does not inhibit the occurrence of œstrus. Zondek (647) has reported similar results.

Analysis of the experimental results from X-rayed animals shows that the œstrus-producing hormone is probably elaborated by the first post-irradiation proliferation from the germinal epithelium in animals sterilized while immature, and by the amorphous tissue of follicular derivation in those sterilized when adult. In the former group the similarity of the proliferation



TABLE 9.—INFLUENCE OF X-RAY STERILIZATION ON THE ŒSTROUS CYCLE.  
(After Parkes.)

No. of Animal	Whole Cycle (average length in days)			Dioestrus (average length in days)			Œstrus (average length in days)		
	Before irrad.	After irrad.	Difference after irrad.	Before irrad.	After irrad.	Difference after irrad.	Before irrad.	After irrad.	Difference after irrad.
AX 1	7.3	11.5	+4.2	5.0	8.3	+3.3	2.3	3.2	+0.9
AX 2	5.6	6.6	+1.0	3.6	4.0	+0.4	2.0	2.8	+0.8
AX 3	5.6	6.8	+1.2	2.6	3.8	+1.2	3.0	3.0	—
AX 7	6.2	9.6	+3.4	4.0	5.7	+1.7	2.2	3.9	+1.7
AX 9	6.7	10.2	+3.5	5.0	6.2	+1.2	1.7	4.0	+2.3
AX 16	6.8	5.5	-1.3	4.1	4.0	-0.1	2.7	1.5	-1.2
AX 17	5.9	5.3	-0.6	3.8	3.3	-0.5	2.1	2.0	-0.1
AX 19	7.0	5.0	-2.0	4.0	2.7	-1.3	3.0	2.3	-0.7
AX 20	7.4	5.7	-1.7	4.9	3.7	-1.2	2.6	2.0	-0.6
AX 23	7.3	4.1	-3.2	5.1	2.2	-2.9	2.1	1.9	-0.2
AX 33	5.2	6.3	+1.1	3.3	3.6	+0.3	1.9	2.6	+0.7
AX 37	4.8	6.5	+1.7	2.5	3.9	+1.4	2.2	2.6	+0.4
AX 41	5.4	5.7	+0.3	2.6	3.3	+0.7	2.8	2.3	-0.5
AX 44	4.6	8.0	+3.4	2.6	6.0	+3.4	2.0	2.0	—
AX 45	5.1	6.0	+0.9	2.9	3.4	+0.5	2.3	2.6	+0.3
AX 46	7.0	6.3	-0.7	4.3	3.7	-0.6	2.7	2.7	—
AX 47	5.5	6.2	+0.7	3.0	3.2	+0.2	2.5	3.0	+0.5
AX 49	6.0	11.7	+5.7	3.0	8.5	+5.5	3.0	3.2	+0.2
AX 51	5.7	5.0	-0.7	3.7	3.0	-0.7	2.0	2.0	—
AX 53	5.0	7.3	+2.3	3.0	5.0	+2.0	2.0	2.3	+0.3
Total	5.98	6.64	+0.66	3.64	4.12	+0.48	2.35	2.52	+0.17

to the so-called interstitial tissue of the ovary suggests a comparison with the supposed endocrine activity of this latter tissue. On the other hand, the irregularity of the occurrence of ovarian interstitial tissue, together with our complete ignorance as to its endocrine significance, makes it difficult to arrive at any conclusions.

*Significance of occurrence of œstrus after follicular ablation.* Allen (12) has doubted whether any real information regarding the normal animal can be obtained from these experiments on sterilized mice. This objection might be of importance if the sterilized mouse were separate and distinct from the normal animal. In animals irradiated when adult, however, the change to the sterilized condition is very gradual. Even after a dose of X-rays which will lead to complete sterility, the degeneration of the follicles is comparatively slow, and during the transition period when the Graafian follicles are gradually disappearing, the œstrous cycle shows no abnormality. Since it is unlikely that the regulation of œstrous periodicity could be taken over imperceptibly by a different mechanism while the sterilization changes are proceeding, it is highly probable that the same periodic mechanism is at work in the sterilized animal as in the normal. Three general conclusions may be drawn from these experiments on the occurrence of the œstrous cycle after X-ray sterilization:

- (a) The Graafian follicle is probably not the essential source of the œstrus-producing hormone;
- (b) The periodicity of œstrus is not governed by the periodic maturation of follicles;
- (c) Since the elimination of the corpora lutea of ovulation has also no effect on the periodicity of œstrus, they can perform no such inhibitory function in the unmated mouse as has been demonstrated by Hammond (265) in the cow and by Loeb (390, 400) in the guinea-pig.

In those animals in which the corpora lutea of ovulation have been shown to inhibit the next œstrous period, it is probable that the elimination of the follicular system would, by indirectly eliminating the corpora lutea, shorten the diœstrous interval. The same result would be expected in the mouse under condi-

tions where pseudo-pregnancy should normally be produced, namely, after sterile copulation. Experiments showed, however that this did not occur in all cases (500). It seems probable that the mechanism concerned in the stimulation of luteal tissue after sterile copulation also affects the tissues of the sterilized ovary. Some X-rayed mice, when mated, will actually copulate every four or five days, namely, at the periodicity which is characteristic of œstrus in the unmated mouse. In the majority, however, this is not found, and after one or two œstrous periods attended by copulation, the œstrous cycle gradually fades out.

That the Graafian follicle is not necessarily the essential source of œstrin is emphasized by quantitative examination of the liquor folliculi and of the residual tissue of the ovary after removal of all large follicles (see p. 107). This examination has shown that œstrin is fairly equally divided between the follicles and the stroma (but see also 613), and it is obvious that the stroma has at least as good a claim to be considered the site of origin of œstrin as has the follicular tissue. The X-ray work reveals the ovary in quite a new light, and two main problems are raised:

- (a) If follicular maturation does not initiate œstrus, by what means can the synchronization be arranged ?
- (b) How is the periodicity of œstrus regulated ?

*Periodic action of œstrin.* It is possible that œstrin is produced at intervals and so achieves its periodic action. It must be remembered, however, that the œstrus-producing hormone can be extracted from the ovary even when œstrus is completely in abeyance, as during anœstrus and pregnancy. If, as seems probable, the production of œstrin by the ovary is continuous, some periodic mechanism, such as the cyclic attainment of a threshold value, must exist. It has also been suggested that the accessory organs have a periodic increase and decrease in sensitivity to the œstrus-producing hormone. This supposition was based on the idea that continuous injection of œstrin would not prolong the œstrous changes indefinitely. More recent work, however, has shown that cornification can be prolonged at will and therefore that no periodic uterine sensitivity occurs (see

p. 126). The difficulties in the way of explaining the periodicity of œstrus on the ground that the ovary regulates its own periodicity, complicated the explanation of the results of X-ray sterilization. Recently, however, the discovery of the pituitary-ovary mechanism has made possible a simpler explanation.

*Synchronization of œstrus and follicular maturation.* It was originally thought from the X-ray experiments that the œstrus-producing hormone caused the maturation of the follicle as the œstrous change in the ovary, in the same way as it caused œstrous changes in the accessory organs. This possibility was supported by the discovery that in the mouse the maturation growth of the follicle does not start until the œstrus-producing stimulus has become active (see p. 136). On this view the œstrous changes in the accessory organs and the maturation of the follicle would be synchronized by their both being due to a common stimulus, namely the œstrus-producing hormone. It has not been possible, however, to substantiate this hypothesis, owing to the fact that follicular maturation cannot be induced by the injection of the œstrus-producing hormone at times when it would not otherwise occur, such as during the lactation diœstrus, during pseudo-pregnancy, or during pregnancy (see p. 119).

#### (d) OCCURRENCE OF FOLLICULAR MATURATION WITHOUT ŒSTRUS

The opposite result to the occurrence of œstrus after follicular ablation, *i.e.* the occurrence of follicular maturation without œstrus, has been very ingeniously produced by Zondek (647), who, by feeding thallium to mice, was able to suppress all cyclic changes in the accessory organs. This suppression probably means that the production of œstrin was inhibited; but nevertheless the histological ovarian cycle was unaffected, ovulation and the formation of corpora lutea taking place. The maturation of the Graafian follicle in these animals, therefore, cannot have been dependent upon the production of œstrin. It is thus possible to reach the conclusion that the production of œstrin is not dependent upon the maturation of the Graafian follicles, and, conversely, that the maturation of the Graafian follicles is



not dependent upon the production of œstrin. Zondek (647) draws attention to this absence of correlation as follows: 'Wir haben also festgestellt: das Ei beherrscht nicht das Hormon, das Ovarialhormon beherrscht aber auch nicht das Ei. Ei und Ovarialhormon stehen nebeneinander, sind koordiniert, sind gleichberechtigt. Sie stehen aber unter der Herrschaft einer zentralen Regulation. . . .'

All this initial work paved the way for the discovery of the influence exerted on the ovary by the anterior pituitary body, and it is possible to assert definitely at this stage that the regulation of ovarian periodicity is controlled by the anterior pituitary, and that ovarian regulation is therefore external to the ovary itself. The same conclusion is indicated by numerous experiments on ovarian grafts.

(e) REASONS FOR SUPPOSING OVARIAN REGULATION  
TO BE EXTERNAL

The hypothesis that the periodicity of ovarian endocrine activity depended upon the periodicity of its cyclic structures seemed so well-founded that small attention was originally paid to the facts which implied that the regulation of the ovary might be to some extent external. Recent work on the mechanism of the œstrous cycle, however, has brought these experiments into prominence.

*Ovarian grafts.* As long ago as 1900 it was shown by Foa (207) that extraordinary effects were obtained by grafting the ovaries from one animal into an animal of a different age. He found that the ovary of the immature animal grafted into an ovariectomized adult underwent rapid development and attained a state of maturity long before it would have done so in its original environment. Many experiments have been made in confirmation of Foa's original observations (365, 367), and further details have been added. Converse experiments have shown that an adult ovary grafted into an ovariectomized immature female loses both its histological and endocrine cyclic activity. Control operations have shown quite adequately that neither effect is due to manipulation, and it must be concluded, therefore, that the ovarian age is regulated by that of the soma. Parallel

results are obtained when an ovary is grafted into a castrated male, though where a mature ovary is grafted into a mature male, the periodicity of the ovary is lost and a state which has been compared to persistent œstrus appears. Hammond (264) sums up these facts by stating that 'the age of puberty is determined by the nutritive state of the soma of the animal and not by age changes in the ovary itself.'

*Compensatory hypertrophy of the ovary.* It has been known for many years that the removal of one ovary will result in the immediate hypertrophy of the remaining ovary. This hypertrophy usually proceeds to a degree which makes possible the maturation of the usual number of follicles (35, 42, 115, 148). It is possible to obtain the same result after removing the whole of one ovary and the greater part of the other. Thus, a small ovarian fragment will undergo extensive hypertrophy and in time produce as many follicles as would both original ovaries. This process of hypertrophy appears to be limited only by the supply of oocytes. Lipschütz (375) has shown quite definitely that after a time hypertrophied ovarian tissue may become almost denuded of oocytes owing to the comparatively small number left in the original fragment. This observation is very strong evidence against the view that germ-cells may be re-formed in the adult female. The hypertrophy of ovarian fragments is paralleled by the hypotrophy of superfluous ovarian tissue. An extra ovary grafted into an animal does not function—three ovaries produce only the same number of mature follicles as do two ovaries.

The fact that an animal will ripen the normal number of follicles from minute amounts of ovarian tissue shows definitely that some limiting factor is at work to prevent the wholesale maturation of follicles from the normal ovaries. This has been expressed by Lipschütz (370) as the 'law of follicular constancy.' In the same way the endocrine activity of one ovary becomes as efficient as that of the initial two ovaries. Thus, the length of cycle after unilateral ovariectomy has been shown (425, 491, 599) to be indistinguishable from the normal.

*The 'generative ferment.'* Heape (288) put forward the hypothesis that some substance required for both growth and reproduction is present in the body in such small amounts

that only one process can proceed at a time. Hammond (264) has tentatively accepted this hypothesis to explain the maturation of immature ovaries grafted into the mature animal, and the mechanism of compensatory hypertrophy of the ovary. On this view, the hypothetical substance in question, to which the name 'generative ferment' was given, is used in the young animal for growth, none being available for the reproductive processes, which are thus in abeyance. When body growth stops, however, the substance can be utilized by the reproductive organs, and their activity begins. Similarly, the substance may be regarded as essential for follicular maturation, and the amount present at any one time is only sufficient to ripen a certain number of follicles. When one ovary is removed the entire supply is available for the use of the other, which is therefore able to mature twice the ordinary number of follicles. A similar view has been adopted by Lipschütz (369) and others, who, however, have used the more reasonable term 'X-substances' to denote the hypothetical factor. An extension of this hypothesis has been put forward to explain the absence of ovarian activity during pregnancy, the use of the substance for uterine and foetal growth preventing its use by the ovary. Though purely speculative, this theory of the somatic control of the ovary appears to have received some vindication from recent work on the effect of anterior pituitary substances on the ovary.

## CHAPTER IX

### THE RELATION BETWEEN THE OVARY AND THE ANTERIOR PITUITARY BODY

#### (a) INTRODUCTION

THE observations and experiments recorded in Chapter VIII make it evident that the ovary does not regulate its own periodicity, and it is necessary, therefore, to look to some somatic tissue for the source of this regulation. The evidence suggests that a somatic endocrine organ is responsible.

For many years some connection has been assumed between the gonads and the other endocrine organs: thus in experimental and clinical studies, the thyroid, thymus, adrenals, and pituitary body have all shown some correlation with the ovary and testis. The evidence is not sufficiently definite to indicate that the thyroid (656), thymus (444), or adrenals (154) could be the seat of the regulation of ovarian periodicity. On the other hand, very striking experiments have recently demonstrated a close relationship between the anterior pituitary body and the ovary.

Various authors, including Fröhlich (234) and Cushing (150), have pointed out that disorders of the anterior pituitary result in marked aberration of sexual function, as well as in bodily abnormalities such as gigantism. Both hypo- and hyper-pituitarism have been described in association with amenorrhœa and infantile sex organs in the human female. The experimental attack on the problem is comparatively recent, and may be said to have begun with Evans' (182) observations on the effect of pituitary extracts on the ovary, and with Smith's (575) work on the effects of hypophysectomy on the œstrous cycle.



## (b) LUTEINIZATION OF THE GRAAFIAN FOLLICLE

Evans (182) found that large amounts of a saline extract of ox anterior pituitary, when injected daily into the normal rat, resulted in the disappearance of the œstrous cycle during the whole time that injections were carried out. This suppression of œstrus was found to be associated with remarkable changes in the ovary. All Graafian follicles which had reached the size at which the antrum appears had undergone luteinization, namely, had formed corpora lutea atretica without the intermediate act of ovulation. As the result of this, the ovaries of the injected animals came to consist mainly of a large mass of corpora lutea in which were embedded the remains of the ova. At the cessation of injection the œstrous cycle returned after varying periods of time. It has since been shown (502, 610) that the luteal tissue produced as a result of this treatment is remarkably healthy and will perform all the functions normally associated with the corpus luteum.

*Preparation of extract.* Evans' early extracts were merely made with saline, but his technique was subsequently improved and elaborated as follows (610): Anterior lobes of ox pituitaries were carefully dissected out, washed, and partially sterilized in 70% alcohol. The tissue was then ground in a mortar and extracted over-night on ice with 0.1 *N* sodium hydroxide. After extraction, the macerated preparation was neutralized to phenol red with 0.2 *N* acetic acid. After centrifuging, the supernatant fluid was used for injection in amounts of about 1 c.c. per day, equivalent to about 1 gm. of original tissue. The extracts were injected intraperitoneally by Evans and his collaborators, and their results have been criticized on the grounds that the effects may have been due to the introduction of irritating material into the peritoneal cavity. It has been shown, however, that the same result can be produced by subcutaneous injection (500), and adequate controls with other tissues have shown quite definitely that the luteinizing effect is due to some principle of the anterior lobe of the pituitary.

*Histological effects.* The first histological effect on the ovary of the injection of such extracts is swelling of the follicular epithelium, with corresponding crushing in of the antrum

(500). The ovum itself then shows atrophic changes, such as fragmentation of the nucleus and general shrinkage. The continuing hypertrophy of the follicular epithelium gradually closes the cavity of the follicle, until the latter becomes solid. Mitoses are not observed during this process and the increase in



FIG. 51.—OVARY OF ADULT MOUSE INJECTED WITH SODIUM HYDROXIDE EXTRACT OF ANTERIOR PITUITARY BODY.

Large numbers of atretic corpora lutea are present, and few follicles.

the amount of tissue can be accounted for solely by the growth, mainly cytoplasmic, of the individual cells. Allowing for the restricted space and the presence of liquor folliculi and the ovum, the process appears to be analogous with that normally occurring after ovulation. The corpora lutea atretica thus formed are crowded closely together, but do not become confluent; they remain as discrete bodies having definite lines of demarcation. Since the action of this type of extract depends in the normal



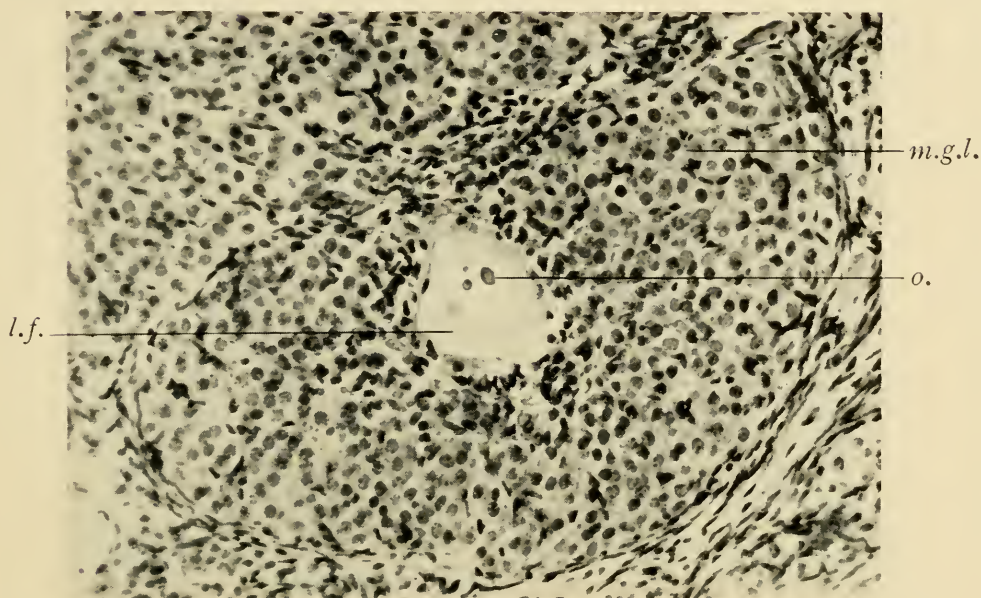


FIG. 52.—EARLY STAGE IN LUTEINIZATION OF THE GRAAFIAN FOLLICLE OF MOUSE BY SODIUM HYDROXIDE EXTRACT OF ANTERIOR PITUITARY BODY.

*l.f.* liquor folliculi; *m.g.l.* membrana granulosa undergoing luteinization; *o.* remains of ovum.

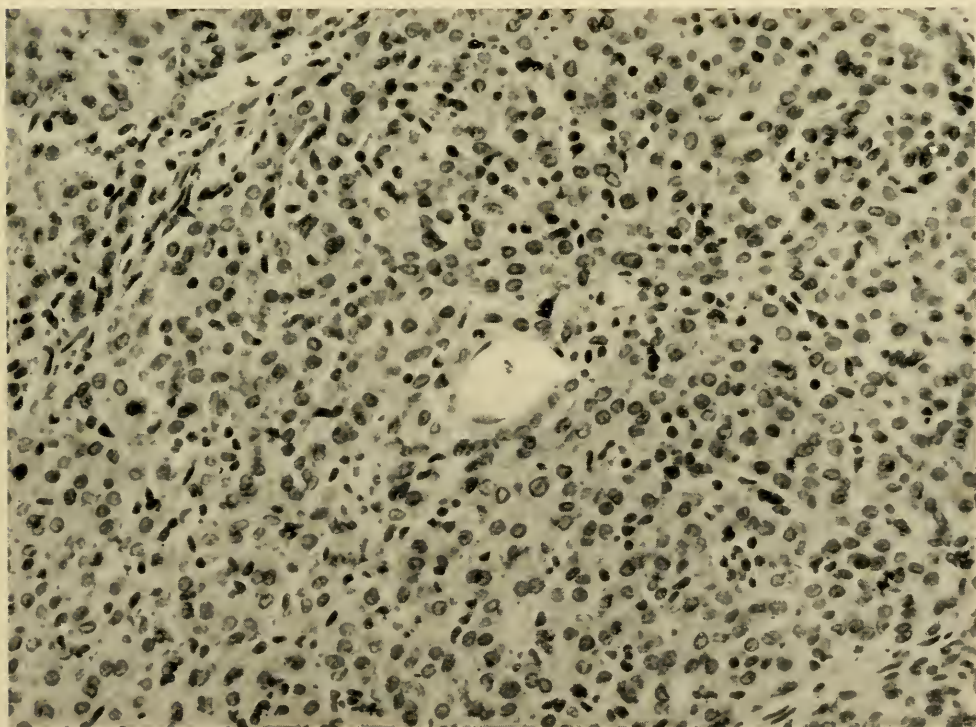


FIG. 53.—LATER STAGE OF LUTEINIZATION.  
(*cf.* fig. 52).

animal upon the presence of follicles, it was originally thought that injection of an animal sterilized by exposure to X-rays

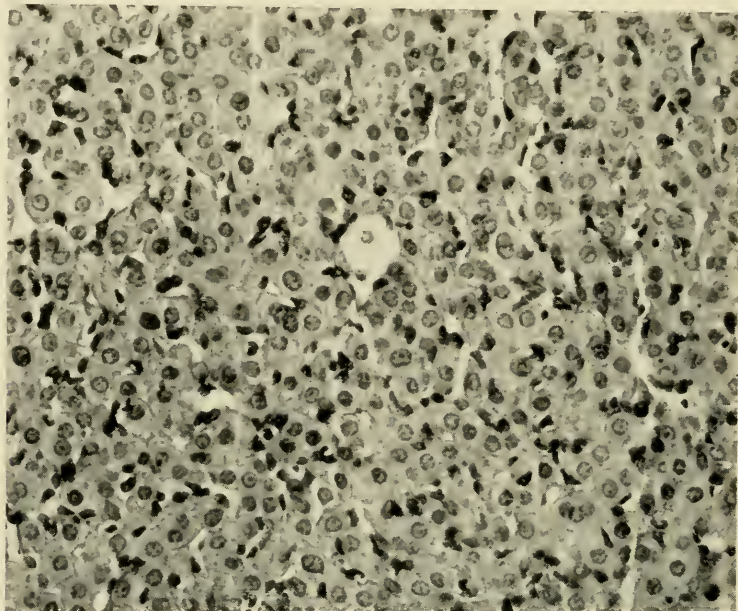


FIG. 54.—ATRETIC CORPUS LUTEUM IN UNTREATED MOUSE.  
(*cf.* fig. 52 ).

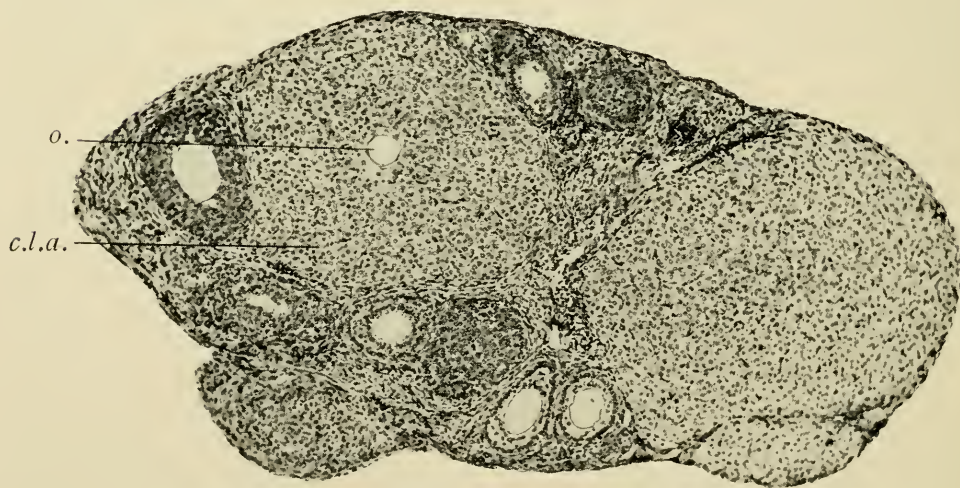


FIG. 55.—OVARY OF MOUSE INJECTED WITH SODIUM HYDROXIDE  
EXTRACT OF ANTERIOR PITUITARY AT THREE WEEKS OLD.  
*c.l.a.*, corpus luteum atreticum; *o.* remains of ovum.

would not cause the typical inhibition of œstrus, in other words, would not produce luteinization of the ovary. This expectation,



however, was not confirmed (500). Injection of the sterilized mouse possessing no Graafian follicles results in the luteinization of the whole of the first post-irradiation proliferation (see p. 140), and in the consequent inhibition of œstrus in the accessory organs. This histological alteration of the ovary was



FIG. 56.—OVARY OF RABBIT INJECTED WITH SODIUM HYDROXIDE EXTRACT OF ANTERIOR PITUITARY, SHOWING LARGE NUMBERS OF ATRETIC CORPORA LUTEA.

not permanent, however, and the cessation of injection was followed by the reversion of the luteinized X-rayed ovary to the normal X-rayed type and by the recurrence of œstrus.

The action of these pituitary extracts has also been investigated in the rabbit. In this animal, also, atretic corpora lutea are produced on a large scale, but in many cases blood follicles are formed (502). Many of the atretic corpora lutea are incompletely solidified and contain a fluid centre consisting of blood and liquor folliculi. In spite of these slight differences in histo-

logical effect, the usual characteristic luteal activity is found in the ovaries of injected rabbits.

In the immature animal similar luteinization of the follicles occurs, neither ovulation nor œstrous changes in the accessory organs taking place. Brouha (88) found that the action of œstrin in the immature animal is not materially inhibited by such luteinization.

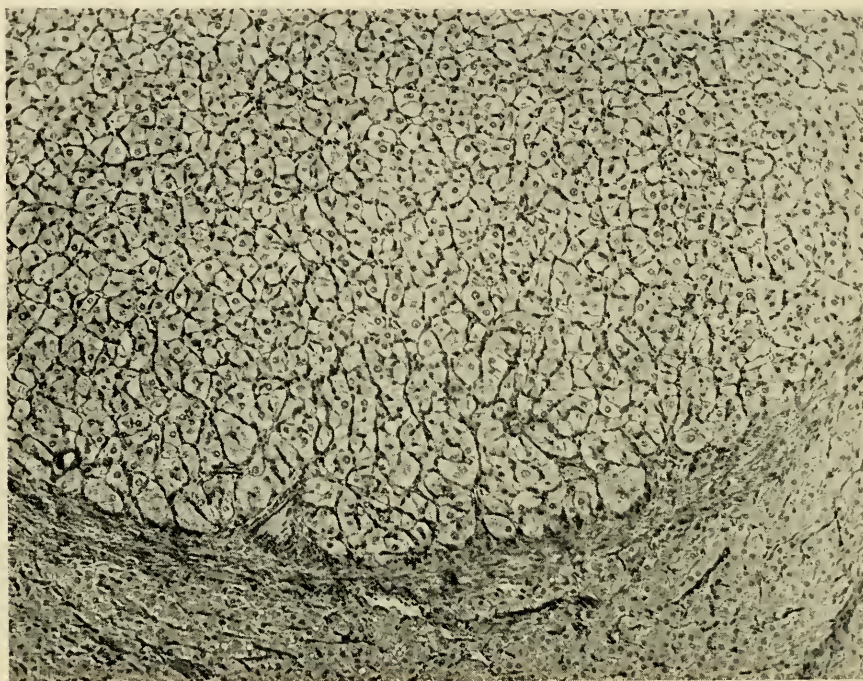


FIG. 57.—LUTEAL TISSUE OF OVARY OF FIG. 56, SHOWING HEALTHY CONDITION.

### (c) THE PRODUCTION OF OVULATION

The amount of evidence suggesting that the ovary does not regulate its own periodicity led Zondek and Aschheim in 1927 (656) to carry out most thorough researches on the effects of other endocrine organs and body tissues upon the female reproductive organs. As a result of these researches they came to the following conclusions:

- (a) Implantation of male or female anterior pituitary into the immature mouse brings about precocious œstrus, including the ovarian changes.



(b) The effect is exerted purely through the ovary, which is caused to undergo precocious maturation and to elaborate the œstrus-producing hormone.

At about the same time, Smith (575), having found that hypophysectomy in the rat stopped the œstrous cycle, had begun to investigate the effects of 'implantations' of the pituitary body

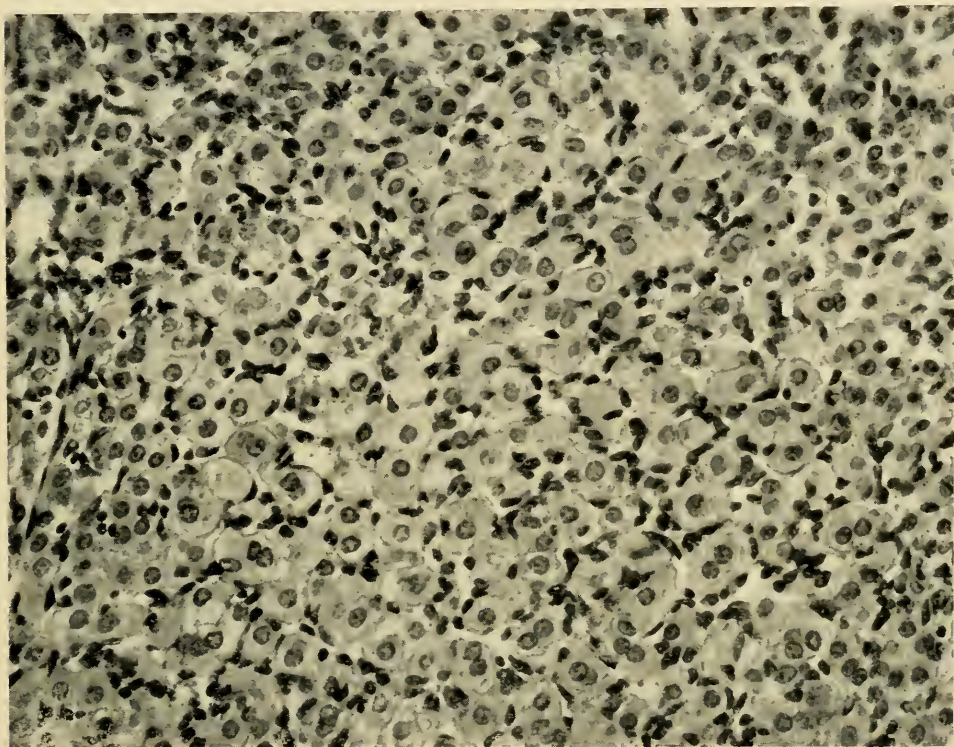


FIG. 58.—LUTEAL TISSUE PRODUCED IN STERILIZED OVARY OF MOUSE BY ANTERIOR PITUITARY PREPARATIONS (*cf.* fig. 48).

(576-9). As in Zondek and Aschheim's experiments, the implants were not grafts, but rather intramuscular injections of macerated tissue. Subcutaneous injection of saline suspensions of anterior pituitary tissue have the same effect. Later Smith, in collaboration with Engle, arrived at results substantially the same as those of the German workers, *i.e.* that implantation of anterior pituitary tissue into the immature animal causes the rapid appearance of the uterine, vaginal, and ovarian changes characteristic of œstrus. The duration of treatment necessary is inversely proportional to the age of the animal. The im-

plants, like the NaOH extracts, have no effect whatever on the accessory organs in the ovariectomized animal. Smith and Engle (581) found that the immature ovary after implant-

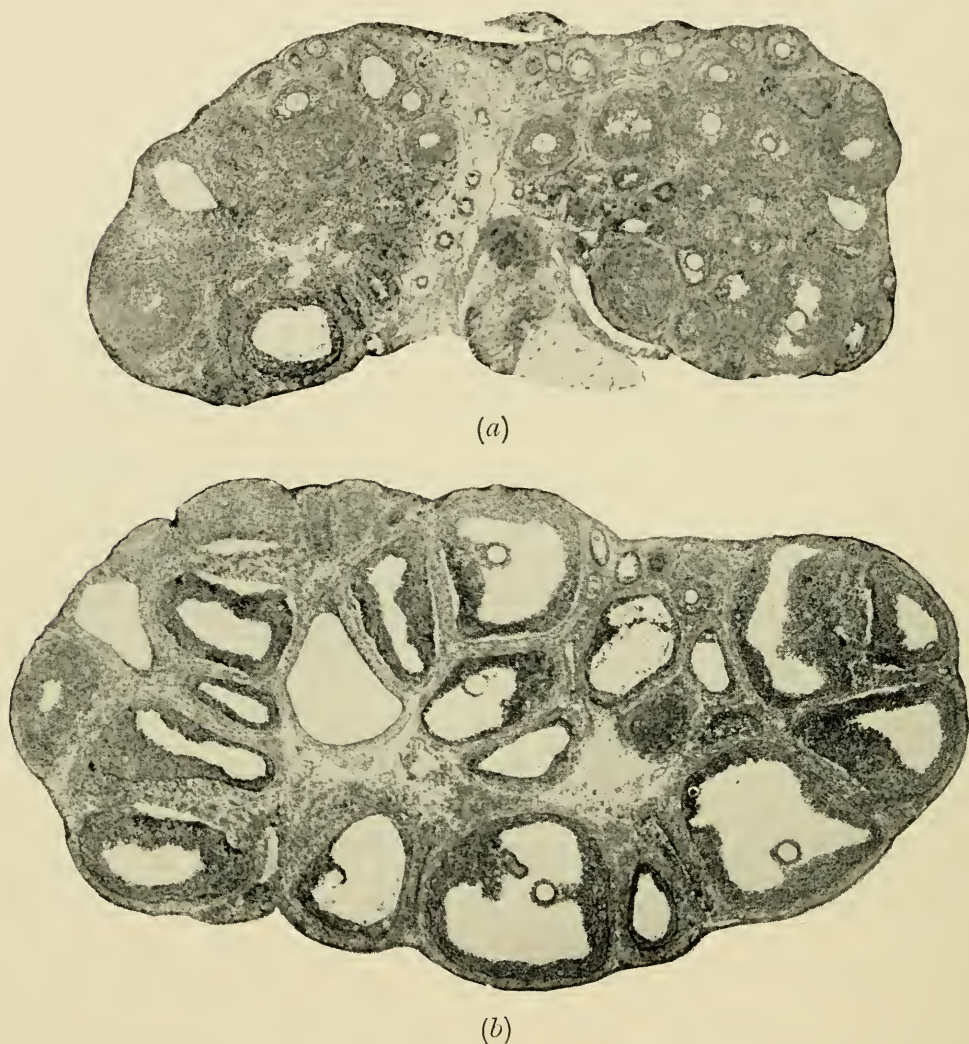


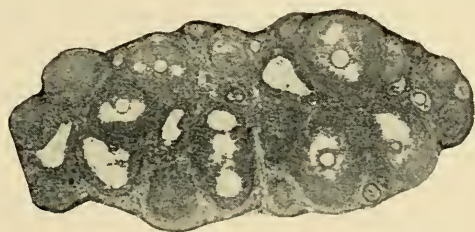
FIG. 59.—EFFECT OF ANTERIOR PITUITARY IMPLANTS ON OVARY OF IMMATURE RAT.

(a) control litter mate; (b) follicular maturation induced on twenty-ninth day by eight daily implants of two mouse hypophyses (From Smith and Engle).

ation of anterior pituitary might be ten times as large as the control in the rat or nineteen times in the mouse. This was not due to any increase in size of mature follicles as compared with the normal adult, but to a great increase in their number and in that of the corpora lutea produced by ovulation.



The result of injection of suspensions of the anterior pituitary tissue is therefore essentially the rapid maturation of Graafian follicles in the ovary, with the coincident appearance of œstrous



(a)



(b)

FIG. 60.—EFFECT OF ANTERIOR PITUITARY IMPLANTS ON OVARY OF IMMATURE RAT.

(a) Control litter mate; (b) Mature follicles and corpora lutea found after four daily implantations of anterior lobe of rabbit. (From Smith and Engle.)

changes in the accessory organs. The production of œstrin by the stimulated immature ovary in such experiments has been attributed to the hypertrophy caused in the Graafian follicles, but the

same effect has been observed (500) when the injected immature animals have been sterilized by exposure to X-rays and therefore possess no Graafian follicles. In such cases the production of œstrus, and therefore of œstrin, must have been dependent on the stimulation of the tissue of the irradiated ovary. In the ovary of the sterilized adult, no histological changes were

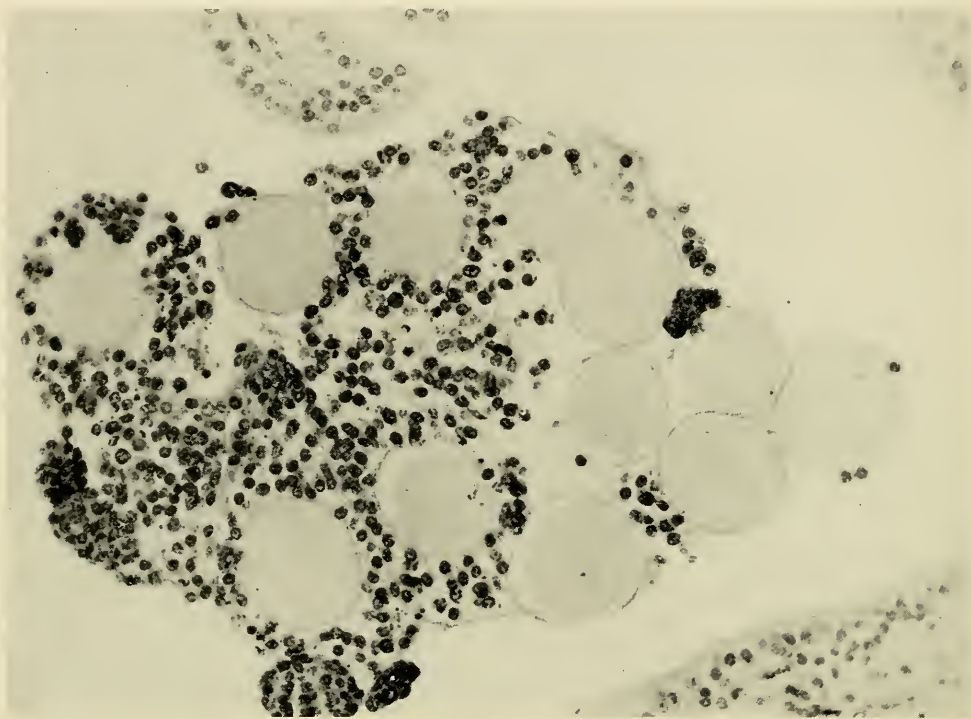


FIG. 61.—GROUP OF TUBAL OVA FOLLOWING SUPER-OVULATION DURING PRECOCIOUS ŒSTRUS IN THE MOUSE.

Forty-eight ova were present in this tube. (From Smith and Engle.)

observed following the injection of anterior pituitary suspensions, but the duration of cornified vaginal smears was prolonged.

The only important difference between the work of Zondek and Aschheim and that of Smith and Engle seems to be that whereas the latter authors always found that normal follicular maturation occurred, the former found that atretic corpora lutea and blood follicles were also produced. Smith and Engle (581) actually state: 'In contrast to the large number of follicles which are found undergoing atresia in the normal animal, we

rarely find any atretic follicles in the precociously matured animals.' According to Fels (206) the formation of atretic corpora lutea is more frequent than ovulation. In view of the earlier work of Evans on the luteinizing effects of anterior pituitary this distinction is of considerable interest. The problem of whether one or two anterior pituitary substances are involved is discussed below.

The pioneer work of Zondek and Aschheim, and of Smith and Engle has now been confirmed by many authors, including Fels (206), Brouha and Simonnet (100), Loewe and co-workers (423) and Siegmund (564). Brouha and Simonnet, however, consider that another pituitary substance, fat-soluble and producing œstrus in castrated animals, can be demonstrated.

(d) ARE TWO ANTERIOR PITUITARY SUBSTANCES  
CONCERNED IN THE REGULATION OF THE OVARY?

The difference in the effects following injection of Evans' sodium hydroxide extract and injection of the macerated fresh tissue, led to the tentative supposition that two different anterior pituitary principles were involved, one causing the conversion of the follicular granulosa to luteal cells and another causing the burst of growth preceding follicular maturation. Doubt has recently fallen on this supposition. In the first place, the method of obtaining the two preparations is fundamentally different. (a) The macerated suspensions are fresh and correspond only to minute amounts, 5 to 20 mgms., of fresh tissue daily; the sodium hydroxide extracts, on the other hand, made from ox pituitaries, may not be really fresh and the daily amount injected corresponds to about 1 gm. of original tissue. (b) The later preparations used by Evans (182) were subjected to a fairly drastic chemical treatment, namely, extraction with sodium hydroxide. His earlier extracts, however, were only made with saline; and similar suppression of ovulation has been obtained by Walker (629) who administered the fresh substance to fowls.

More serious criticism of the view that two anterior pituitary substances are involved is forthcoming from the work of Zondek and Aschheim (656). These authors, in investigating the effects



of implants or extracts on the immature animal, found that three ovarian reactions were characteristic of this treatment.

- (a) In the majority of cases follicles matured and ovulated, as found by Smith and Engle.
- (b) Other follicles, however, in the same ovary, became atretic and underwent luteinization without ovulation in a manner corresponding essentially with that described by Evans.
- (c) A small number of follicles in the same ovary would become cystic and appear as 'Blutpunkte' on the surface of the ovary.

Zondek considers these results to show that only one anterior pituitary hormone is involved, and that the complete luteinization caused by the sodium hydroxide extracts is due to the injection of relatively large amounts, while the characteristic ovulation effect of the suspensions results from the substance being present in comparatively small amounts. Zondek's aqueous extract of anterior pituitary can apparently produce the three reactions he describes in any proportion, according to the amounts administered.

Evans and Long (186) failed to produce œstrus in the immature rat by the injection of the alkali preparation. This would be expected, since Zondek and Aschheim (658) found that the ovulation-stimulating principle is destroyed by alkali. On the other hand, if there are two anterior pituitary substances affecting the ovary, the mixed ovulation and luteinization obtained by Zondek and Aschheim might be due to the presence of both substances in the implants. This would, however, apply equally to Smith and Engle's preparations.

With the evidence available at present, it is difficult to decide whether two different substances are concerned in these effects, but basing a conclusion on Evans' results with alkali extracts, and Zondek and Aschheim's observation that the ovulation-producing substance is destroyed by alkali, it seems not improbable that two are concerned, as supposed by Bellerby (58). There is little doubt that the problem will soon be cleared up by administration of (a) the NaOH extracts in smaller amounts, (b) the two types of preparation simultaneously.



Since recent detailed work on the relation between the anterior pituitary body and the ovary has been largely concerned with the production of ovulation, the following account will be restricted to this aspect of the problem.

(e) ASSAY OF ANTERIOR PITUITARY EXTRACTS

It is essential that anterior pituitary extracts should be tested on the intact animal, as contrasted with œstrin which must be assayed on the ovariectomized animal. The effects of œstrin

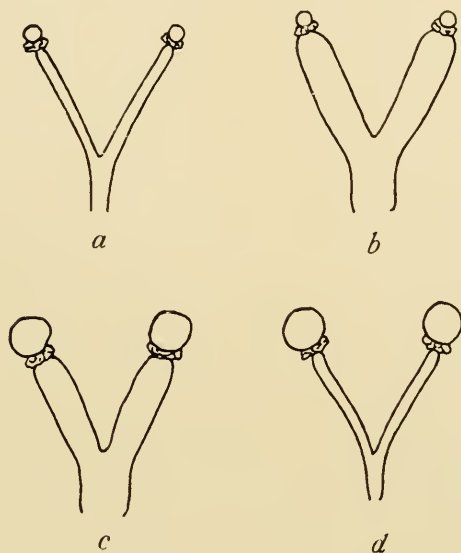


FIG. 62.—EFFECT OF ŒSTRIN AND ANTERIOR PITUITARY EXTRACTS ON UTERUS AND OVARY OF YOUNG MOUSE.

(a) Normal; (b) œstrin—action on uterus only; (c) implants of anterior pituitary—action on ovary and thence on the uterus; (d) NaOH extract of anterior pituitary—action on ovary only.

and anterior pituitary on the intact immature animal are superficially similar, *i.e.* the production of precocious œstrus. Actually, of course, the result is quite different. Œstrin, acting directly on the accessory organs, causes the reaction in the ovariectomized animal; anterior pituitary preparations, primarily affecting the ovary, will act only on the intact animal.

When testing for the anterior pituitary substance in fluids which might contain œstrin (*i.e.* the urine of pregnancy), Aschheim and Zondek (39) give a control dose to ovariectomized animals, and, if this is negative, test again on intact



animals. They use the 6-8 gms. immature mouse, and give six doses over forty-eight hours. The result appears in about 100 hours. They define the mouse unit of anterior pituitary hormone as the amount which given in six doses will produce precocious œstrus, including the ovarian changes, in the 6-8 gms. mouse within 100 hours.

(f) PREPARATION AND PROPERTIES

Zondek and Aschheim have obtained aqueous extracts of the anterior pituitary body which have the same effect as the implants. Further, they have described (658) the preparation from urine as follows: The urine is acidified with acetic acid, filtered, and evaporated down to half the volume. It is then extracted with ether to remove œstrin. The watery residue is dialysed and evaporated to dryness. By further purification (details not given) a whitish amorphous water-soluble powder is obtained. These authors give a table of the comparative chemical properties of œstrin and the anterior pituitary substance (Table 10).

TABLE 10.—COMPARISON OF PROPERTIES OF ŒSTRIN AND THE ANTERIOR PITUITARY SUBSTANCE.

Property.	Œstrin.	Anterior pituitary substance.
Thermostability	Very stable	Destroyed at 60°C.
Resistance to acid and alkali	Very resistant	Easily destroyed
Solubility in water	Soluble	Soluble
Solubility in lipoid solvents	Very soluble	Insoluble

Smith (577) has reported that anterior pituitary substance is inactive when administered by mouth; probably the active principle is destroyed by the digestive enzymes.

(g) DISTRIBUTION OF THE ANTERIOR PITUITARY HORMONE

After the discovery of the characteristic effects of the injection of anterior pituitary preparations, attempts were soon made to

discover whether other tissues and body fluids possessed the same active principle. Zondek and Aschheim (658) found that the implantation of 0.1 gm. placenta produced ovulation, while blood serum and urine of pregnancy were active in amounts of 0.5 c.c. and 1-2 c.c. respectively. The blood of a pregnant cow was found to contain even larger amounts (659). Zondek and Aschheim thus suppose that elaboration of the hormone is more rapid during pregnancy, and since it is absent in the urine of the

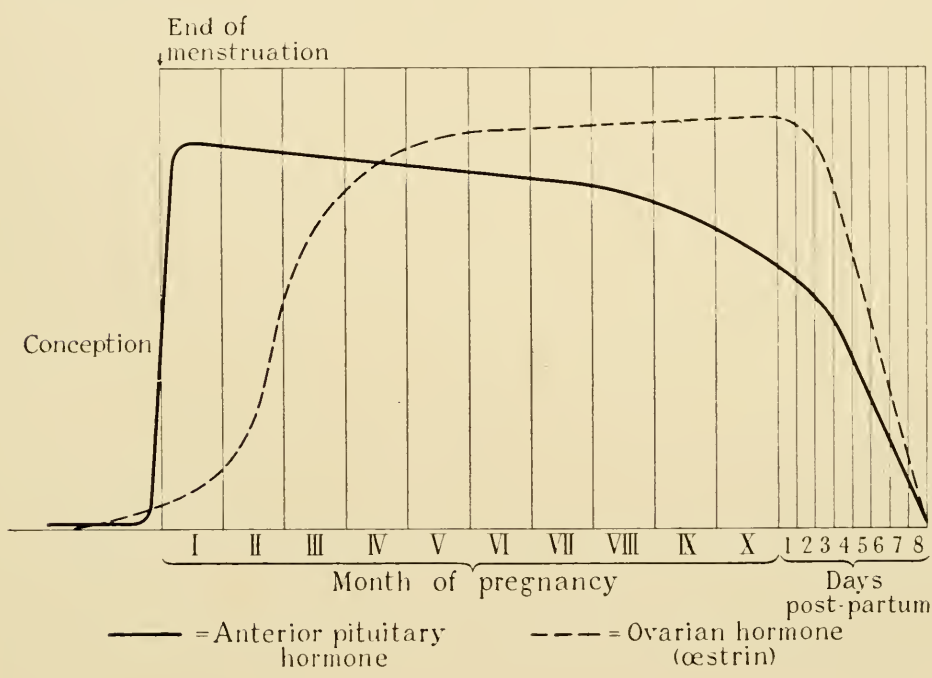


FIG. 63.—DIAGRAM OF AMOUNT OF ŒSTRIN AND ANTERIOR PITUITARY SUBSTANCE IN THE URINE OF PREGNANCY.  
(From Aschheim and Zondek.)

non-pregnant female, its occurrence in the urine of early pregnancy is used as a test of this condition in the early stages (39). By means of their technique (see p. 165) for separating and testing the anterior pituitary hormone and œstrin in the urine, they are able (40) to give the relative concentrations in the urine during the course of pregnancy as shown in Table 11 and fig. 63. Fels (204) also reports the hormone in the blood during pregnancy. The significance of the occurrence of this large amount of anterior pituitary substance in the urine of pregnancy is not as yet understood.

Aschheim and Zondek (38) have also detected the anterior pituitary hormone in the decidua, in the corpus luteum of pregnancy, in navel blood, and in tubal mucous membrane.

TABLE II.—ŒSTRIN AND ANTERIOR PITUITARY HORMONE  
IN URINE OF PREGNANCY.

(After Aschheim and Zondek.)

Stage	Œstrin m.u. per litre	Anterior pituitary hormone m.u. per litre
1-8 weeks	300-600	3000-5000
3-7 months	5000-7000	3000-6000
7-10 months	6000-10000	2000-3000

Negative results were given by a large number of other tissues and fluids, including male urine.

#### (h) ACTION ON THE NORMAL ANIMAL

*Prenatal period.* The anterior pituitary hormone would appear to be unable to traverse the placenta—otherwise, in view of its abundance in the blood during pregnancy, the foetuses would presumably be born in a state of sexual maturity.

*Prepubertal period.* Smith and Engle (581), using mice rather younger than those used by Zondek and Aschheim, have secured the ovulation reaction at fifteen days of age, five days after the beginning of injections. As a result of the treatment, follicles mature rapidly in the ovary and the antrum appears, together with the cumulus oophorus. Ovulation then takes place and corpora lutea are formed. Smith and Engle imply that the follicles and corpora lutea are all normal, but according to Zondek and Aschheim atretic and blood follicles are also formed. These ovarian changes result in the elaboration of œstrin and in the production of œstrous changes in the accessory organs. Owing, presumably, to the formation of corpora lutea, continued administration does not lead to persistent œstrus, but great hypertrophy of the accessory organs is produced. The continued injection of both anterior pituitary



substance and œstrin does, however, lead to prolonged œstrus (658). Zondek and Aschheim found that mice in premature puberty will not mate owing to disparity of size. Smith (580), however, reports that they will copulate at nineteen days old if precocious puberty has been induced. Anterior pituitary implants have a stimulating action on the testis of the young male, with consequent acceleration of growth in the accessory organs (626).

*Adult animal.* Smith and Engle (581) have described the effect of the anterior pituitary substance on the adult animal. 'Super-ovulation' of large numbers of follicles takes place in the ovary, and œstrous changes occur in the accessory organs, followed by an interval in which an irregular type of vaginal smear is found. The ovaries of injected animals are found to contain large numbers of corpora lutea, but these differ essentially from the corpora lutea produced by sodium hydroxide extracts in that they are normal corpora lutea resulting from normal maturation and ovulation of Graafian follicles. Prolonged dosage will lead to the disappearance by ovulation of all large-sized follicles from the ovary.

Smith and Engle and also Zondek and Aschheim have found very numerous true corpora lutea in ovaries of animals thus treated, and the former workers have found up to forty-eight ova in one Fallopian tube. Engle (177), however, found, in the early stages of pregnancy, twenty-nine embryos at most after such super-ovulation. Much thinning out must take place both before and after implantation.

Marrian and Parkes (439) have found that the anœstrous period brought about in the rat by inanition or vitamin B deficiency may be terminated by an œstrous period following the administration of anterior pituitary substance. The typical ovarian and extra-ovarian changes were produced.

*The pregnant animal.* Zondek and Aschheim (659) and Engle and Mermod (179) have described the effects of administering anterior pituitary substance during pregnancy. The latter found that pregnancy could be readily interrupted in the middle third, though less easily later on. Ovulation occurred soon after abortion, but not if pregnancy continued. Zondek and Aschheim found that although abortion could be caused, with

suitable dosage ovulation might be induced without terminating gestation. It is possible that ovulation may be caused by amounts of anterior pituitary insufficient to produce enough œstrin to cause abortion. The abortion induced by anterior pituitary implants is clearly comparable with that produced by œstrin injection (see p. 118).

*The senile animal.* Zondek and Aschheim (658) have reported that œstrus can be induced in the senile mouse after the cessation of the cycle by anterior pituitary treatment. In this case the result differs essentially from that of œstrin injection (see p. 119) in that the ovary is stimulated to ovulation. Similar results on mice showing spontaneous ovarian deficiency have been reported by Loewe, Voss and Pass (423).

#### (i) THE MECHANISM OF OVARIAN REGULATION

*Influence of the anterior pituitary on the ovary.* The work described above makes it evident that the anterior pituitary body produces a substance or substances which have a most potent action upon the ovary, and it is thus reasonable to suppose that the anterior pituitary plays some part in regulating the normal ovarian cycle. The fact that precocious œstrus can be induced in the ovaries and accessory organs of the normal immature animal by administration of anterior pituitary substance, suggests that the first œstrus of puberty is brought about by some action of the pituitary. This, in itself, would not explain how the first œstrus is precipitated: the problem is merely transferred from the ovary to the anterior pituitary. Since anterior pituitary bodies from male or female, young or mature animals are all efficacious, it is difficult to explain how the first stimulus to the ovary is liberated from the anterior pituitary. Smith and Engle (581) 'believe that the hypothesis of the periodic liberation of gonad-stimulating hormone of the pituitary may explain the periodic ripening of groups of follicles more satisfactorily than any previously advanced.' Whether one or two substances are secreted by the anterior pituitary for the regulation of the ovary is still uncertain, but if two occur it would seem that the first is connected with the maturation of the follicle and the second with the transformation of the

ruptured follicle into luteal tissue. If one substance only is involved, quantitative variation probably produces the different effects.

It has been shown by Engle (178) that the hypertrophy of the remaining ovary after unilateral ovariectomy is greatly expedited by the injection of anterior pituitary substance. The author considers this result as evidence that the factor limiting the number of follicles ovulated at any one time, the factor governing the law of follicular constancy, variously called 'generative ferment' or X-substance, is merely the follicle-stimulating principle of the anterior pituitary.

*Influence of changes in the accessory organs on the anterior pituitary.* It is necessary to mention here some theoretical considerations upon which no work has been carried out. If the anterior pituitary is directly responsible for the changes in the ovary, then some means must exist whereby events in the accessory organs can influence the anterior pituitary. For instance, since ovulation does not take place during pregnancy, some mechanism must cause the anterior pituitary at this time to stimulate the corpus luteum and not the follicle. Similarly, it must be concluded that the absence of œstrus during pseudopregnancy in the mouse indicates that the anterior pituitary body reacts to sterile copulation and exerts a stimulating effect upon the corpora lutea. Since the effect of sterile copulation can be produced by mechanical irritation of the uterine cervix, it would seem that such stimulation can react upon the anterior pituitary.

A similar conclusion is reached by another argument. The fact that cervical stimulation will activate the corpora lutea in a grafted ovary in the rat (425) makes it fairly certain that the effect is not direct. The intermediate point, where the stimulus changes from nervous to endocrine, may reasonably be supposed to be the anterior pituitary. In the rabbit copulation probably causes ovulation (see p. 54) by stimulation of the anterior pituitary. Since the action of copulation can only be nervous in nature, ovulation in the rabbit would appear to occur as the result of a vulva-pituitary-ovary chain of stimulation, in which the first link is nervous and the second endocrine.

Similarly, the fact that lactation in the rat and mouse

causes the corpora lutea of the immediate post-partum ovulation to persist means presumably that lactation influences the anterior pituitary body, causing it to exert the luteinizing stimulus. Then again, premature weaning apparently causes the anterior pituitary body to exert its stimulus to follicular maturation.

*Influence of events in the ovary on the anterior pituitary.* It is well known that the ovarian cycle can be expedited by various means such as puncturing the maturing follicles (478) or removing young corpora lutea (265). This might theoretically be explained in either of two ways:

- (a) That the alteration in the ovary has expedited a cycle in the anterior pituitary.
- (b) That anterior pituitary substance is made available for corpora lutea by the destruction of follicles, and vice versa.

The latter view would presuppose the presence of only one anterior pituitary substance, and also the absence of endocrine cycle in the anterior pituitary.

The occurrence of the cycle in X-ray sterilized animals shows that it is not brought about by follicles and corpora lutea alternately utilizing a single anterior pituitary substance. Hence, hypothesis (b) is invalidated and hypothesis (a) must be accepted. Thus, cyclic endocrine activity exists in the anterior pituitary, even if only one substance is produced. It is evident, therefore, that events in the ovary may, under certain conditions, influence the cycle in the anterior pituitary.



## CHAPTER X

### THE INTERNAL SECRETION OF THE CORPUS LUTEUM

#### (a) INTRODUCTION

THE facts discussed in previous chapters make it clear that the ovary possesses some periodicity which is quite independent of the periodic production of Graafian follicles and of corpora lutea, and which is probably under the control of the anterior pituitary body. This basic periodicity appears in the unmated cycle of the normal mouse, because in this animal the complete ablation of the periodic ovarian structures does not alter the periodicity of œstrus. In most species, however, even in the unmated animal, this basic cycle is disturbed by the transient development of corpora lutea after each ovulation, namely, by the interpolation of a luteal phase. When pregnancy leads to the full development of the corpora lutea the derangement of the cycle is much greater. The conditions in animals, such as the rabbit and ferret, in which œstrus persists in the absence of mating, are somewhat difficult to explain by this conception of the ovarian function; it is necessary to suppose that the factor which causes periodic œstrus in animals such as the rat and mouse is persistently operative in the unmated rabbit and ferret.

*The functions of the corpus luteum.* The analysis of the mechanism controlling the luteal phase of the sexual cycle has not yet proceeded as far as the analysis of the mechanism of the follicular phase. Nevertheless, a great deal of work has been carried out on the functions of the corpus luteum, and, in spite of the present tendency to minimize its importance in the œstrous cycle, certain definite functions can be ascribed to it. The subsequent history of the corpus luteum formed after

ovulation depends both on the species of animal in question, and also upon the occurrences which take place in the accessory organs. In the short five-day cycle of the unmated rat and mouse it may be asserted that the corpus luteum performs no function. After sterile copulation, however, in these animals, the corpora lutea, now the corpora lutea of pseudo-pregnancy, undergo a greater development and the postponement of the next œstrous period for about twelve days is correlated with the development of other luteal functions during this pseudo-pregnant period. Both the rat and the mouse, for instance, develop sensitivity of the uterus. This is characteristic of the activity of the corpus luteum and is always found in the normal cycle in the guinea-pig, and also during lactation in the rat and the mouse. In the guinea-pig a prominent luteal phase corresponding to the pseudo-pregnant period in the rat and the mouse occurs in the ordinary unmated cycle. In the Eutheria, the corpora lutea attain their maximum growth and their full functional activity during pregnancy.

In the rat and mouse, therefore, the history of the corpus luteum of ovulation depends upon (a) whether copulation has taken place, and (b) whether copulation is fertile and followed by the implantation of embryos. In the rabbit and ferret, no corpora lutea are found until copulation has taken place; the corpora lutea then undergo great development, resulting in a period of pseudo-pregnancy which is much more obvious than that in the guinea-pig, rat, or mouse. In the dog, where ovulation is spontaneous at œstrus, a well-marked development of the corpus luteum also takes place quite irrespective of pregnancy, and a definite pseudo-pregnant period is found. There exists, therefore, a reciprocal co-ordination between the accessory organs and the corpora lutea. In the rat and mouse the corpus luteum of ovulation needs the stimulus provided by the act of copulation before it can develop to a functional stage. Further, as in all higher mammals, it requires the stimulus provided by implantation of embryos before developing to the fully mature state. Following parturition in the rat and mouse, lactation causes the corpora lutea of the post-partum ovulation to become persistent for a period of about three weeks. The nature of the stimulus exerted by

these occurrences in the accessory organs is not precisely known, but the anterior pituitary body is presumably concerned (see p. 171). Having been stimulated, the corpus luteum elaborates the internal secretion which performs its various functions.

Many experiments have been carried out on the experimental ablation and stimulation of the corpora lutea, and as a result of these it is possible to state that four functions are performed by the corpora lutea of pregnancy, pseudo-pregnancy, or lactation. These functions may be summarized as follows:

- (a) The inhibition of ovulation and of œstrous changes in the accessory organs.
- (b) The sensitization of the uterus for the implantation of fertilized ova.
- (c) The development of the mammary glands from the condition in which they are found at œstrus to that characteristic of the end of the luteal phase.
- (d) The maintenance of pregnancy.

*Methods of removing the corpora lutea.* Experimental work on the functions of the corpora lutea has dealt mainly with the effects of their removal. The exact methods by which this has been achieved have varied considerably and some importance attaches to this point. Most workers have performed double ovariectomy in order to remove the corpora lutea, but this method has the great disadvantage that all ovarian activity is eliminated; it is therefore quite useless in work dealing with the return of œstrus. In large monotocous animals, such as the cow, the corpora lutea can readily be squeezed out from the rest of the ovary, but in smaller animals, such as the rabbit, surgical dissection or cauterization has to be employed if the corpora lutea only are to be eliminated. Such an operation is very severe and may easily lead to post-operative effects which may be confused with those due to ablation of the corpora lutea. As regards the operation during pregnancy, for instance, several workers have found that their control experiments (cutting the ovary, etc.) gave almost the same results. In an animal as small as the mouse even these methods are impracticable. The ideal subject for experiments of this nature would be a small monotocous animal in which the ovary containing the corpus luteum

could be readily removed, leaving intact the second ovary containing no corpus luteum, to carry on the other ovarian functions. Recently a technique has been elaborated whereby the mouse can be converted to this type (499). This technique consists in unilaterally sterilizing the young animal by X-rays. When adult, corpora lutea are present only in one ovary, which can be removed surgically without any adverse after-effects. The other ovary, without corpora lutea, is capable of carrying on the ovarian functions other than those associated with the corpora lutea (see p. 143).

#### (b) INHIBITION OF OVULATION AND ŒSTRUS

The idea that the corpus luteum performs the function of suppressing ovulation during pregnancy appears to have been put forward originally by Beard (54) and by Prenant (520). These authors based their conclusion on the general functional correlation which is known to exist between the development of the corpus luteum and the absence of œstrus. This correlation, of course, is not found during the period of anœstrus, throughout which ovarian activity, both follicular and luteal, is in abeyance. This, however, is a special condition, and so far as the ordinary ovarian cycle of the regular polyœstrous animal is concerned, the persistence of the corpus luteum is invariably associated with the absence of œstrus.

A few authentic cases of superfœtation have been recorded (see Smith, 571). These seem to show that ovulation may occur during pregnancy, but the condition is very rare, and in the normal animal the presence of a functioning corpus luteum prevents ovulation. The exact extent to which follicular maturation is inhibited by the functional corpus luteum seems to show specific variation. In the guinea-pig, for instance, Loeb (389) describes waves of follicular growth even during pregnancy. This growth ends, however, in atresia, inhibition of ovulation occurring in all species. During recent years the hypothesis put forward by Beard and Prenant has been extended by a variety of experimental studies, the more important of which are summarized below.

*Functional correlation.* In certain animals, such as the cow



and the guinea-pig, the removal of the corpora lutea of ovulation expedites the appearance of the next œstrous period. Thus, Loeb (390) found that their removal in the guinea-pig led to the appearance of the next œstrus at about the fourteenth day instead of at the twentieth day. Loeb (400) also found that the removal of the corpora lutea during pregnancy did not always result in the immediate termination of gestation. Ovulation, however, very soon followed the operation whether or not the fœtuses remained temporarily.

Hammond (265), by squeezing out the corpus luteum from the ovary of the cow, was able to cause the next œstrous period to occur at the ninth day instead of the twentieth day. In the mouse (494), however, indirect elimination of the corpora lutea by exposure to X-rays does not bring about an earlier appearance of the next œstrus. This is due to the fact that in this animal the diœstrous interval is very short, and practically no development of the corpora lutea to a functional stage takes place in the ordinary unmated cycle. In other words, the unmated mouse possesses no luteal phase in the cycle, and therefore the elimination of the corpora lutea cannot suppress such a phase. After sterile copulation, however, when the cycle in the mouse does possess a luteal phase, the elimination of the corpora lutea hastens the reappearance of œstrus. Thus, in certain mice, ovulation may temporarily be in abeyance at œstrus so that no corpora lutea are formed. In such animals pseudo-pregnancy is not found (500). In the cow and the guinea-pig the luteal phase is prominent and its elimination, therefore, leads to the earlier appearance of the next œstrus.

Similar conclusions may be drawn from converse experiments, namely, from the experimental prolongation of the functional life of the corpus luteum. Correlated with this there is found a prolonged disappearance of œstrous changes both in the ovary and in the accessory reproductive organs. Thus, Loeb (401) found that hysterectomy in the guinea-pig causes the corpora lutea to remain intact and functional for a long period. This condition is accompanied by a cessation of the œstrous cycle. In the cow various workers, including Hess (298), Williams (638), and Tandler (608), have found that the persistence of corpora lutea results in sterility, owing to the suppression of ovulation.

The expulsion of such abnormally long-lived corpora lutea usually brings about the return of œstrus. In the same way the human cycle may cease when the corpora lutea persist abnormally and the removal of such abnormal corpora lutea is followed, according to Ochsner (482), by the return of the cycle. Quite recently it has been found possible to prolong the functional life of ovarian luteal tissue almost indefinitely by the injection of sodium hydroxide extracts of the anterior pituitary body. Thus, in the mouse and rat the immense production of luteal tissue which follows such treatment is associated with the complete absence of œstrus and ovulation, while in the X-rayed animal the luteinization of the tissue of the sterilized ovary by similar treatment also brings about the suppression of œstrus.

*Mechanism of œstrus inhibition.* Definite information is lacking as to how the corpus luteum brings about the suppression of œstrus. It is clear that it cannot be merely a local mechanical effect in the ovary itself; the presence of a corpus luteum in one ovary is sufficient to inhibit the œstrus-producing activity of both ovaries. In the cow and other usually monotocous animals only one corpus luteum at a time is normally present. In polytocous animals, the same condition can be produced experimentally by eliminating the Graafian follicles, and hence the corpora lutea, of one ovary by exposure to X-rays.

*Preparation of œstrus-inhibiting extracts.* By analogy with other ovarian functions, it is probable that the œstrus-inhibiting action of the persistent corpus luteum is brought about by some endocrine activity. Only very recently, however, have extracts been prepared from the corpus luteum which have any genuine œstrus-inhibiting activity. Corner and Hurni (129) reported negative results from the injection of rats with corpus luteum preparations, while Loeb (400) working on the guinea-pig, was unable to produce regularly positive effects. Pearl and Surface (512) claim to have succeeded in stopping ovulation in laying hens by injection of extracts of a commercial preparation of corpora lutea, while Kennedy (320) reported positive results on the rabbit by the injection of saline extracts of similar material. Pearl and Surface, however, state that toxic effects were produced by their extracts and the same appears probable in Kennedy's experiments from the fact that ovulation was

suppressed in some animals for months after the end of treatment. Clearly no physiological action comparable with the normal activity of the corpus luteum can have caused such a prolonged inhibitory effect. Haberlandt (259), however, has more recently described œstrus-inhibiting effects from the injection of extracts of both ovary and placenta. In the rabbit, the inhibition and recovery is described by him as consisting of three stages: (*a*) complete inhibition of both ovulation and mating instincts, (*b*) inhibition of ovulation, though copulation will take place, (*c*) restoration of full ovulation and mating instinct. Since a rabbit in good condition will copulate during pseudo-pregnancy and pregnancy, one would imagine that toxic effects also influenced these experiments. The necessity for considering the possible toxicity of tissue extracts has been emphasized by Köhler (334), and by Herrmann and Stein (295), who obtained inhibition of œstrus by the injection of irritant organic substances.

Loewe (417) has reported the inhibition of œstrus in the mouse by injection of a commercial extract of corpus luteum. Quite recently various workers have concentrated on the preparation of corpus luteum extracts capable of causing the suppression of œstrus in the normal animal. Papanicolaou (487) injected lipid extracts into the guinea-pig and brought about the suppression of œstrus for a considerable period. No method of preparation, however, was given by this author. Johnston and Gould (316) were unable to inhibit the action of œstrus-producing extracts by the simultaneous injection of extracts of corpus luteum. On the other hand, Parkes and Bellerby (507) inhibited œstrus in the unmated mouse by the injection of extracts of corpus luteum made with fat solvents. Corpora lutea of the cow were dissected and all hollow specimens rejected. The tissue of the solid corpora lutea was then minced and ground up with anhydrous sodium sulphate. The mixture was extracted with ether two or three times in the cold, the ether extracts evaporated down to small bulk, and acetone added to precipitate the phosphatides. The acetone extract, when evaporated down, gave a brownish oil which, emulsified with  $\frac{1}{2}$  % sodium bicarbonate and injected subcutaneously, was found to be active in inhibiting œstrus. Large amounts of this oil had



to be injected to produce positive results, but control injections showed that the administration of even larger amounts of inert fat emulsions had no effect on the cycle.

Beginning work from another viewpoint Hisaw (303) was able to prepare extracts of corpus luteum which had the remarkable property of dissolving the pubic ligaments of the guinea-pig and the pocket gopher. This extract was afterwards found to have other properties characteristic of luteal activity. Thus, its injection into the normal animal inhibited œstrus and produced the sensitivity of the uterus to mechanical stimulation. The improvement of the extraction of the active substance from the corpus luteum is still being carried on by Hisaw and his co-workers, but the most recent information shows that their extract is prepared essentially as follows. The corpora lutea of the sow are used, preferably those of a pregnant animal. The solid tissue only is employed and after grinding in a mortar, twice the volume of acidified ethyl alcohol is added (98 c.c. of 95 % alcohol—2 c.c. of HCl). The mixture is shaken thoroughly and allowed to stand for twelve hours. The alcohol is then decanted and a second extract made. This second extract is removed by means of a press and combined with the first extract. The alcoholic extract may be used as a stock solution and keeps well. In the further stages of purification, the alcohol is filtered and evaporated in a vacuum at a low temperature. The aqueous residue after removing the alcohol is neutralized to a pH. of 5.4 with a 15 % solution of NaOH. A heavy precipitate is formed which should be filtered off and re-extracted. The salts present may be removed by dialysis.

Gley (243) has described the preparation of an œstrus-inhibiting extract by the following method. Corpora lutea of the cow are extracted with tartaric acid and the extract treated with lead acetate. This brings down various toxic substances, but not the hormone, which is water-soluble. Further purification is effected with  $\text{Cu}(\text{OH})_2$ , which is subsequently removed by  $\text{H}_2\text{S}$ . After neutralization the solution is protein free and may be injected. It causes congestion of the uterus and suppression of œstrus. Payne, Peenan, and Cartland (511) have described the preparation of an œstrus-inhibiting substance from corpora lutea by saponification.



*Distribution of the œstrus-inhibiting hormone.* According to Hisaw (304), the corpus luteum is the primary source of the œstrus-inhibiting hormone, but it may be detected in the blood of the pregnant rabbit, guinea-pig, sow, cat, dog and mare, and in the maternal and foetal sides of the rabbit placenta.

*Properties of the œstrus-inhibiting hormone.* Although little precise experimental work has yet been performed, it is possible to deduce from the methods which give active extracts certain of the fundamental properties of the œstrus-inhibiting hormone. There can be little doubt that the active principle is soluble in organic solvents such as alcohol, and there is good reason for supposing that its thermo-stability is low. Hisaw (304) states that the resistance to acid and alkali is poor, while Parkes and Bellerby's original extracts seem to show that oxidization is very rapid. It is fairly evident, therefore, that this œstrus-inhibiting hormone is not easy to handle, and its delicate nature probably accounts for the failures of early investigators to obtain active extracts.

*Action of the œstrus-inhibiting hormone.* The ovaries of animals in which œstrus has been inhibited are described by Papanicolaou (487) as showing complete absence of corpora lutea, and the presence of a large number of medium-sized follicles, in which the theca interna was enlarging preparatory to atresia. The action of the hormone on the ovary and uterus has been studied by Hisaw and co-workers (305) and Gley (244). The former state that a condition analogous to that found during pseudo-pregnancy is set up in the uterus. Gley, on the other hand, describes effects similar to those following ovariectomy. This result, however, is highly improbable if a true corpus luteum effect is being reproduced.

*Assay of the œstrus-inhibiting hormone.* Papanicolaou (487) suggested that the œstrus-inhibiting hormone should be assayed by its power to inhibit œstrus in the guinea-pig for a period of five days, one unit being the amount required to do this. Such a method, however, would clearly be both inaccurate and cumbersome. Since it is not possible to say precisely when the next œstrous period of an animal is due, the degree of inhibition of this period cannot be gauged precisely. Hisaw (304) and his co-workers assay their extract by its ability to relax the pubic

ligaments of the virgin guinea-pig in full œstrus. The smallest amount that will do this is taken as a unit. Fifteen to twenty of these units are required daily to inhibit œstrus in a rat. It seems probable, however, that the most satisfactory way of assaying the œstrus-inhibiting hormone will be to test it against a known quantity of the œstrus-producing hormone, but this, of course, would involve the accurate assay of the œstrus-producing substance and it cannot be said at the moment that such accuracy has been achieved.

*Scope of function.* It has been found by Weichert (633) that the œstrus-inhibiting hormone also performs the function of sensitizing the uterus to mechanical stimulation (see p. 184), and it is thus possible that the same hormone is responsible for all functions of the corpus luteum.

*The interaction of œstrus-producer and œstrus-inhibitor.* The work described above shows that results are being obtained which indicate that the effects of luteal activity may be reproduced by extracts. The œstrus-inhibiting action of the corpus luteum would thus appear to be definitely endocrine in nature. In this case the interaction of the œstrus-producing hormone and the œstrus-inhibiting hormone affords a rich field for experimental work. Preliminary research in this direction has been carried out by a number of workers, who have injected the œstrus-producing hormone during the time when the corpus luteum dominates ovarian activity, as for instance, during pregnancy or pseudo-pregnancy. In such experiments it has been shown that the injection of the œstrus-producer in sufficient amounts will override the inhibiting action of the persistent corpus luteum. Smith (573), and Parkes and Bellerby (504) were able to override the activity of the persistent corpora lutea of pregnancy in the mouse by the injection of the œstrus-producing hormone, while Engle and Mermod (179) produced the same result by injection of the œstrus-stimulating extracts of anterior pituitary. Some doubt exists as to whether the effects produced were due to overriding the œstrus-inhibiting power of the corpus luteum of pregnancy, or to local action on the uterus. It is hardly possible, therefore, to claim these experiments as demonstrating the interaction of the corpus luteum and the œstrus-producing hormone. The ordinary diœstrous period of the rat and mouse

is so short that no adequate time is available for experiment and the same applies in a lesser degree to the pseudo-pregnant period. During the prolonged inhibition of œstrus which is found during lactation there is, however, an adequate opportunity for experimental work. Parkes and Bellerby (505), studying the effects of injection of the œstrus-producing hormone into the lactating mouse (see p. 117), showed that a very considerable œstrus-inhibiting action is set up by lactation.

By means of two other experiments, inhibition was found to be directly due to the persistent corpora lutea of lactation. In the first, lactating mice were ovariectomized and the amount of œstrin required to produce œstrus determined; the ovariectomized lactating mouse required but very little more œstrin to produce œstrus than the ordinary ovariectomized mouse. In other words, the œstrus-inhibition set up by lactation had disappeared following ovariectomy (505), showing that the inhibition is set up through the ovary and is not merely due to the heavy drain upon the metabolism which must result from lactation. Since the suckling mouse produces between one-fifth and one-quarter of its own weight per day of milk (505), this drain upon the suckling mouse is enormous. The second set of experiments was performed on unilaterally sterilized mice (500). Such mice were allowed to become pregnant and to suckle their litter in the ordinary way. During lactation, the ovary containing the corpora lutea was removed and the sterilized ovary without corpora lutea left. This sterilized ovary (see p. 143 and p. 175), is capable of carrying on all ovarian endocrine functions other than those performed by the corpus luteum. In the lactating mouse containing the sterilized ovary only, the œstrus inhibition was negligible. These experiments showed clearly that the œstrus-inhibition set up by lactation is performed through the corpora lutea, which are caused to become persistent by the act of lactation.

#### (c) SENSITIZATION OF THE UTERUS

*Post-œstrous development of the uterus.* The classic work of Fraenkel (208-11) on the rabbit made it known that the presence of the corpus luteum is necessary for the attachment of the



fertilized ovum to the uterine mucosa, and also for the subsequent maintenance of foetal nutrition. Fraenkel's work was confirmed and extended by the experiments of Ancel and Bouin (29-32) on the same animal. This dependence of the ovum on the influence of the corpus luteum for attachment is clearly correlated with the post-ovulative changes which occur in the uterine endometrium. These changes may be either very obvious histologically, as in the dog, ferret, and rabbit, or they may be less perceptible and indicated mainly by physiological sensitivity, as in the guinea-pig. After sterile copulation in the rabbit and the consequent formation of the corpora lutea, the uterus shows growth, vascularization, and particularly glandular increase in a manner comparable to the growth changes during pregnancy. During this pseudo-pregnant period the six folds of the rabbit endometrium proliferate and become so infiltrated with convoluted glands that in cross section they present a fern-like appearance. This typical change during pseudo-pregnancy has been definitely shown to be under the control of the corpus luteum. Thus, Ancel and Bouin (31), by ablation of the corpora lutea after ovulation, prevented the typical pseudo-pregnant changes, while O'Donoghue (478), having produced luteal tissue experimentally by puncturing the Graafian follicles, was able to bring about the pseudo-pregnant changes without the preliminary act of true ovulation. In the dog similar post-œstrous activity takes place in the uterus, and there can be little doubt that this is due to the activity of the corpus luteum, though experimental evidence is lacking. In the same way post-œstrous changes occur in *Dasyurus*, and Corner (122) has described in the uterus of the sow a certain amount of post-ovulation activity.

*Production of deciduomata.* In other mammals the actual histological changes are less obvious, but, nevertheless, in many cases it has been shown that a peculiar sensitivity to mechanical irritation is present. This reaction of the post-œstrous uterine mucosa was originally shown by Loeb (380) in the guinea-pig. This author, by cutting the endometrium, was able to cause the production of large blocks of decidual cells, to which the name placentomata or deciduomata has been given. Loeb (382-3) found that this sensitivity of the post-œstrous uterus was



entirely dependent upon the presence of the corpora lutea and could be prevented by their removal after ovulation. The removal of both ovaries has a similar effect in preventing this response of the uterus to mechanical irritation. Loeb also found that the sensitivity appeared at a certain definite time after ovulation. These deciduomata were entirely reabsorbed before the occurrence of the next œstrous period and

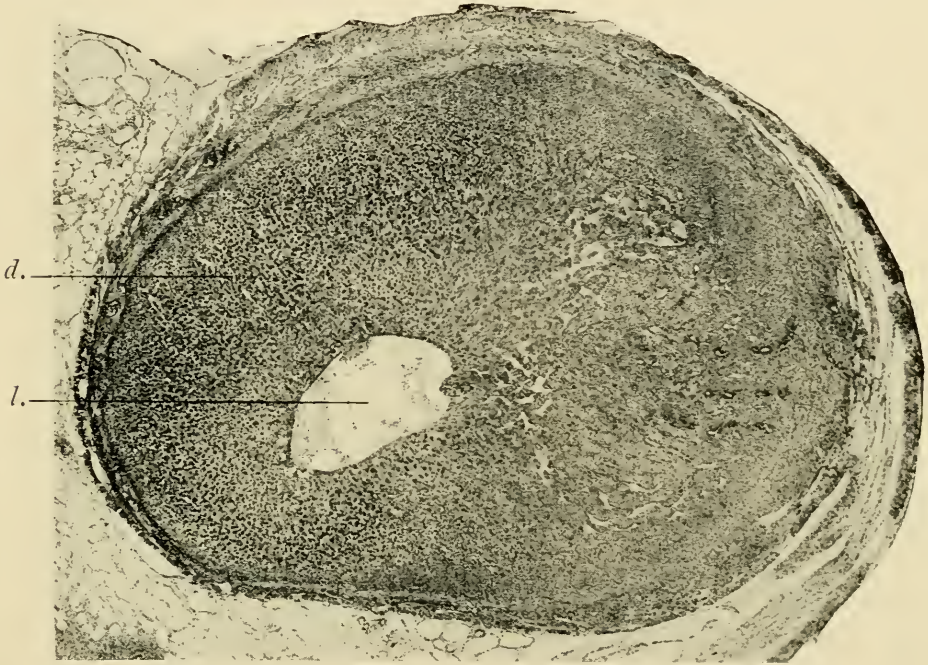


FIG. 64.—UTERUS OF MOUSE (AFTER STERILE COPULATION) WITH DECIDUOMA.

*l.* lumen; *d.* deciduoma.

were shown by Loeb to have no effect upon the length of functional life of the corpus luteum. Pregnancy, however, by prolonging the life of the corpora lutea, prolongs the life of deciduomata. Corner and Warren (130) and Frank (219) were able to produce the same reaction in the rat during lactation. Long and Evans (425), also working on the rat, found that the uterus in this animal was quite unable to respond to mechanical stimulation at any stage of the normal unmated cycle. These authors, however, confirmed the report that the sensitivity did appear in the rat during pseudo-pregnancy and lactation, when the corpora lutea undergo a degree of development not

found in the ordinary unmated cycle. Long and Evans also introduced the technique of inserting a small loop of surgical silk transversely through the uterus as a means of providing the mechanical stimulation. If the stitch passes through the mucosa, the stimulus which it set up is found to be as effective as that of an actual incision through the muscle and mucosa. The uterus was found to be most sensitive in the rat about four days after ovulation. These results have been extended and confirmed by various workers. Loeb (381), Gasbarrini (236), Hammond (263), and Nielsen (474) have induced deciduomata formation during pseudo-pregnancy in the rabbit, while Krainz (335) has obtained a like effect in the bitch. Results agreeing exactly with those of Long and Evans (425) on the rat have been obtained (501) on the mouse, although in this animal the maximum sensitivity occurs somewhat sooner after copulation.

*Evidence of luteal control.* The evidence that the corpora lutea are responsible for this sensitization of the uterus is so strong that its absence during the ordinary unmated cycle in the rat is additional evidence that the corpus luteum of ovulation in this animal does not function. This fact is comparable with the lack of change in the periodicity of œstrus following obliteration of the corpora lutea in the unmated mouse. In the rat and mouse, therefore, the corpus luteum of the unmated animal possesses neither a sensitizing nor an œstrus-inhibiting function. Additional evidence of the luteal control of the sensitization of the uterus has recently been obtained from the fact that sensitivity does not appear even after copulation in sterilized mice possessing no corpora lutea (501). Interesting experiments have been reported by Teel (610) showing that the corpus luteum in the rat is responsible for the sensitization of the uterus. It has been described above how the injection of anterior pituitary extracts will bring about the luteinization of the ovary. Teel was able to show that with this treatment the consequent luteinization of the ovary of the rat made possible the production of deciduomata even in the unmated animal. The deciduomata were produced most readily when the operation was made on the fifth day of injection. The reaction, however, did not occur in the ovariectomized animal and cannot, therefore, have been directly due to the anterior



pituitary extract. There can be little doubt that the sensitivity was induced, when normally it would have been absent, by the

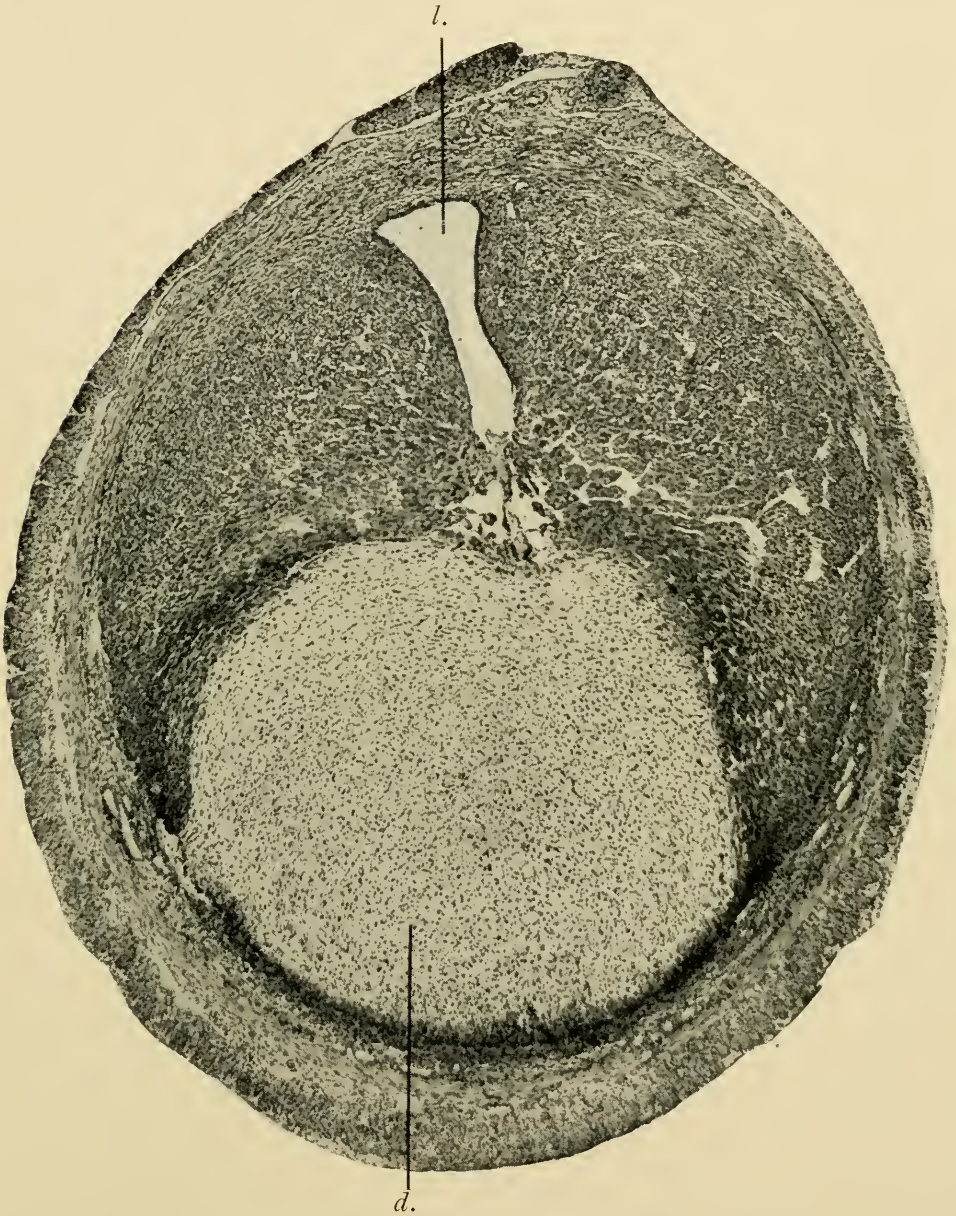


FIG. 65.—UTERUS OF MOUSE WITH DECIDUOMA.

In this case anterior pituitary extract was used to produce the necessary luteal stimulation.

*l.* lumen; *d.* deciduoma.

hypertrophied luteal tissue of the ovary stimulated by the anterior pituitary preparation. Similar results have since been

described for both the rat (87) and the mouse (501). In the mouse the injection of the sodium hydroxide extract of anterior pituitary made possible the production of deciduomata in the unmated animal, but not in the unmated sterilized mouse in spite of the conversion of the irradiated ovary into luteal tissue.

The nature of the activity by which the corpus luteum sensitizes the uterine mucosa is just beginning to be understood. Loeb (383) originally reported two interesting facts, (*a*) that the sensitization is specific to the uterus, other tissues not being affected, (*b*) that the sensitization is equally well induced in grafted uterine tissue. These observations made it clear that the sensitization is chemical in nature, but initial attempts at preparing from the corpus luteum an extract capable of causing this sensitivity, when it would otherwise be absent, were not successful. Loeb (383) obtained only negative results from the injection into ovariectomized animals of corpora lutea extracts and also by the injection of blood from animals in the stage of uterine sensitivity. These results, however, were merely inconclusive. Recently Weichert (633) has been successful in sensitizing the uterus of the rat during the unmated diœstrous cycle by the injection of the œstrus-inhibiting extract of corpus luteum as prepared by Hisaw and his co-workers. A similar result followed injection of corpus luteum extract in the ovariectomized animal, provided that an artificial œstrous period had been first induced by the injection of the œstrus-producing hormone. This work of Weichert is of extraordinary interest in showing that the preliminary activity of the œstrus-producing hormone may be necessary for the later action of the corpus luteum secretion.

#### (*d*) DEVELOPMENT OF THE MAMMARY GLANDS

The facts which have been recorded in previous chapters make it clear that two stages of development take place in the mammary glands even before the appearance of the first corpus luteum. These two stages are (*a*) a slight pre-pubertal development from the time when the female mammary gland differentiates from its male analogue, and (*b*) a burst of growth at the first and subsequent œstrous periods (see p. 130). This growth



still leaves the gland in a rudimentary condition and only during pregnancy (and to a lesser degree during pseudo-pregnancy) does the real mammary development occur.

The initial development of the female mammary glands is clearly endocrine in nature (see p. 78), and the control during pregnancy must also be of the same nature since transplantation of mammary tissue to abnormal sites does not affect its normal development (534). The foetus and placenta, as well as the ovary, have been suggested as possible sources of the stimulus required during pregnancy, but a wealth of evidence has now accumulated to show that the corpus luteum is the responsible factor.

*The mammary gland in pseudo-pregnancy.* After ovulation, correlated with the development of the corpus luteum, an entirely new phase of mammary growth sets in. The extent to which this growth takes place in the non-pregnant animal varies with the species, and with the intensity of the luteal phase in the non-pregnant animal. In the unmated rat and mouse, where the luteal phase of the cycle is missing, growth of the mammary gland appears to take place only at œstrus (see p. 53). Where the luteal phase becomes pronounced, as during pseudo-pregnancy in *Dasyurus*, and in the rabbit and the ferret, very considerable development of the mammary gland takes place even in the absence of foetuses. The withdrawal of the stimulus at the end of pseudo-pregnancy in these animals results in at least a temporary secretion of milk. In the rabbit the development of the mammary glands during pseudo-pregnancy and during pregnancy has been studied in very considerable detail by Ancel and Bouin (30, 32) and Hammond (264). Even when œstrus has lasted for some months the continued activity of œstrus-producing hormone causes no development of the mammary glands other than the slight growth normally associated with œstrus. Immediately ovulation takes place, however, and the corpora lutea are formed, development of the mammary gland begins and even in the absence of pregnancy continues for some fourteen days, that is, as long as the corpus luteum of pseudo-pregnancy is functional. This proliferation consists in the lateral extension and swelling of the ducts. Clumps of alveoli also develop at the ends of the milk ducts.

Ancel and Bouin (30), by removal of the corpora lutea after sterile copulation, were able to show that this growth of the mammary gland during pseudo-pregnancy is entirely dependent upon these structures.

Marshall and Halnan (449) have described the development of the mammary glands of the dog during pseudo-pregnancy; in this animal the constructive phase proceeds so far that the breakdown process at the end of pseudo-pregnancy actually leads to lactation. In *Dasyurus* the development of the mammary glands during pseudo-pregnancy is indistinguishable from that which occurs during true pregnancy; as in other species the growth of the gland is correlated with that of the corpus luteum. In the guinea-pig, according to Loeb and Hesselberg (402), the mammary tissue undergoes very little development during the luteal phase of the ordinary diœstrous cycle in the unmated animal, but there is appreciable growth when the corpora lutea are caused to become abnormally persistent by hysterectomy. During the diœstrous cycle, even when there is a definite luteal phase, as in the guinea-pig, the development of the gland is not normally carried far enough to result in the actual secretion of milk. Woodman and Hammond (644), however, report that virgin heifers after a series of diœstrous cycles may occasionally secrete a small quantity of milk. Dieckmann (159) has described the growth which takes place in the mammary gland during the luteal phase of the human menstrual cycle. None of the changes characteristic of the luteal phase are found after ovariectomy or removal of the corpora lutea. The control of this mammary development during pseudo-pregnancy is known definitely to be endocrine in nature.

Our knowledge of the mammary gland during pseudo-pregnancy makes it evident (*a*) that the presence of foetuses is not essential for at least the initial phases of mammary development, and (*b*) that since the only ovarian change in pseudo-pregnancy is the development of corpora lutea, it is reasonable to suppose that these bodies are the actual site of origin of the stimulus required. The almost synchronized appearance of the katabolic changes in the corpus luteum and in the mammary tissue further supports this view. As a result of his study of the

mammary gland of *Dasyurus*, in which pseudo-pregnancy and pregnancy are of the same length, O'Donoghue (476) came to the conclusion 'that the corpus luteum is a ductless gland producing a secretion which is the inciting cause of the growth of the mammary gland.' Up to the present, however, administration of corpus luteum preparations to the ovariectomized animal has not induced a degree of mammary development comparable to that found in the normal animal during the luteal phase of the cycle. This, however, is doubtless due to the failure of the extracts employed up to the present to contain the active principle. Preliminary experiments with the œstrus-inhibiting extracts of the corpus luteum have given negative results. Loeb and Hesselberg (403) failed to cause mammary development by the injection of aqueous extracts of corpus luteum. Champy and co-workers (119), however, have recently reported the induction of mammary growth by the injection of luteal extracts, while Bencan, Champy, and Keller (59) claim that the corpus luteum substance can be obtained from placentæ as well as from the corpus luteum. The dependence of the mammary gland on the corpus luteum is further shown by experiments on artificial pseudo-pregnancy in the rabbit (Parkes, 502). In these experiments rabbits were injected with the sodium hydroxide extract of anterior pituitary and corpora lutea were caused to appear in the ovary in the absence of copulation. Under these conditions the growth in the mammary gland typical of pseudo-pregnancy occurred.

*The development of the mammary gland during pregnancy.* In the rabbit, the growth of the mammary gland in the first fourteen days of pregnancy is exactly the same as occurs during pseudo-pregnancy. After this period, however, a completely new phase of growth begins, consisting essentially in thickening of the gland as opposed to lateral extension. That a very real difference exists between these two stages of pregnancy is shown by the fact that pseudo-pregnancy in a parous rabbit, in which the ducts are already well developed, produces no greater growth of the gland than pseudo-pregnancy following the first ovulation.

Woodman and Hammond (644), and Asdell (43) have shown that two phases of mammary development occur during preg-



nancy in the cow and the goat. In nulliparæ a great development of the alveoli begins about midway through pregnancy and this is correlated with a change in the type of secretion found in the gland. Before this stage the secretion is of a serous nature and has the characteristics of diluted milk. At the midway stage the secretion changes abruptly to a thick pigmented fluid containing up to 40 % of solids, mostly globulin. Drummond-Robinson and Asdell (171) found that the removal of corpora lutea in the goat did not result in milk secretion, unless the operation was performed after the globulin stage had been reached.

In the guinea-pig, also, two phases of mammary growth during pregnancy have been described by Loeb and Hesselberg (403). For the first twenty-four days of pregnancy (out of sixty) the mammary gland is more or less quiescent (as in the luteal phase of the diœstrous cycle); from this stage onwards continuous growth takes place.

*The cause of the final development during pregnancy.* The initial growth of the mammary gland during pregnancy, corresponding to the growth found during pseudo-pregnancy, is clearly under the influence of the corpus luteum, but for various reasons it has often been thought that the growth taking place during the second half of pregnancy requires some additional stimulus. It has been supposed, for instance, that the corpus luteum does not function during the later stages of pregnancy and cannot, therefore, exert an influence on the mammary gland. It has also been pointed out that the growth of the mammary gland during pseudo-pregnancy, when no fœtuses are present, does not equal that occurring during true pregnancy. For these reasons the theory has been held that some fœtal or other extra-ovarian stimulus is required for the complete growth of the mammary glands. Lane-Claypon and Starling (341) reported experiments on the injection of fœtal and placental extracts into virgin rabbits. These experiments seem to show that some growth could be produced by such means, but their illustrations make it quite clear that the amount of development produced was far less than that normally found during pseudo-pregnancy and consequently less than takes place under the influence of the corpus luteum alone. Ancel and Bouin (33) ascribe the later



development in pregnancy to a uterine myometrial gland. Hammond (264), however, was unable to find such a gland with any regularity. The evidence that no foetal factor is required for the complete development of the glands has always been strong and has recently become conclusive. In the egg-laying mammals where no intra-uterine development of the embryo takes place and where, therefore, no foetal hormone can function, the mammary glands proceed to their full development and function normally. Loeb and Hesselberg (403) found no mammary development in the rare cases where pregnancy persisted for some time after removal of the corpora lutea, but, as these authors point out, abortion finally took place before extensive proliferation would have occurred even in normal pregnancy. Hammond (263) has shown that the presence of decidual tissue during pseudo-pregnancy in the rabbit does not increase the mammary development. The real answer to the question, however, is to be found by prolonging pseudo-pregnancy to the length of normal pregnancy. In the rabbit the slighter mammary development during pseudo-pregnancy, as compared with that during true pregnancy, might be due to the much shorter duration of the former period. In the ferret, where pseudo-pregnancy has the same duration as true pregnancy, the development of the mammary glands during both periods is identical (267). Loeb (401) was able to prolong the life of the dioestrous corpora lutea in the guinea-pig to the duration of pregnancy by the operation of hysterectomy. In such animals, subjected to prolonged luteal action, the mammary glands underwent development comparable, if not quite equal, to that found in pregnancy.

Recently it has been possible to prolong pseudo-pregnancy in the rabbit to the same length as true pregnancy (502). It was thought that this could be done by the continuous injection of the virgin animal with the sodium hydroxide luteinizing preparations of the anterior pituitary body. In practice, however, prolonged administration of such crude preparations affected the animal adversely, and it was found preferable to start the injections at the end of pseudo-pregnancy and to prolong the life of the corpora lutea so as to stretch out pseudo-pregnancy to the length of true pregnancy. Under these conditions, in spite

of the absence of foetuses, the mammary glands undergo the same development as during normal pregnancy. These ex-



FIG. 66.—PHOTOGRAPH OF MAMMARY GLAND OF RABBIT DURING PSEUDO-PREGNANCY PROLONGED BY ANTERIOR PITUITARY EXTRACTS.

Complete growth as found at the end of pregnancy takes place during prolonged pseudo-pregnancy.

periments show quite conclusively that the complete development of the mammary gland can be brought about in the absence of foetuses and is not, therefore, dependent upon any foetal



stimulus. Similar results appear to have been obtained by Grueter (251), though details are not given by this author.

The failure of Loeb and Hesselberg (403) to induce mammary growth by injections of corpus luteum preparations was doubtless due to the mode of preparation (saline suspensions of commercial dried tissue). Where autopsy is performed some days after the cessation of the anterior pituitary injections a copious secretion of milk is found. This shows definitely that

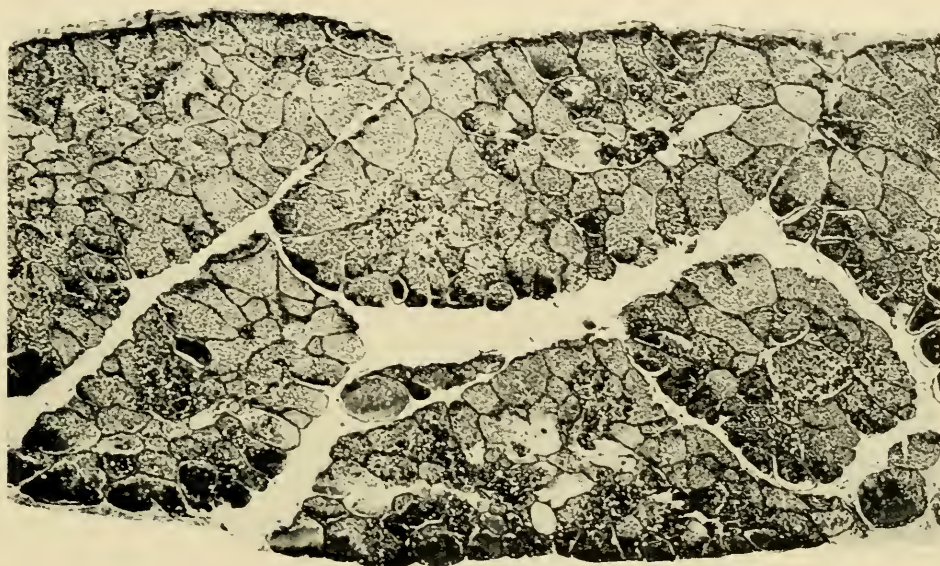


FIG. 67.—SECTION OF GLAND SHOWN IN FIG. 66.

secretion is precipitated by removal of the luteal influence and not by the removal of a hormone present during pregnancy, as postulated by Gaines and Davidson (235).

*Abnormal mammary secretion.* Instances in which the mammary glands function in quite abnormal situations and at abnormal times are not uncommon. The new-born infant of either sex quite often shows some abnormal mammary development leading to the appearance of a certain amount of milk in the ducts. The meaning of this is not entirely known, but it possibly represents some activity on the part of the maternal stimulus operating through the placenta upon the foetus. Secretion by virgin animals is also not unknown (622). More difficult to explain are the cases of mammary secretion in the adult male (152). Many of these cases are doubtless of

problematical authenticity, but at the same time it is possible that some at least are genuine.

#### (e) MAINTENANCE OF PREGNANCY

As pointed out in a previous section there can be little doubt that the corpus luteum is necessary for the sensitization of the uterus, and thus probably for the implantation of ova. Authors are not agreed, however, as to how long the corpus luteum continues to be essential for the maintenance of pregnancy. Fraenkel (211) came to the conclusion that it was only required during the early stages and that subsequently the corpus luteum could sometimes be removed with impunity. Even so, Fraenkel's work showed that the corpus luteum was necessary for some time after implantation had taken place, and, therefore, it was not merely concerned with the sensitization of the uterus. Marshall and Jolly (451) for the dog and the rat, and Kleinhaus and Schenk (324) for the rabbit, came to the same conclusion. Blair Bell (56) and Essen-Møller (181) report clinical cases which suggest that in the human the removal of the corpus luteum of pregnancy during the later stages may produce no adverse effect. Ask-Upmark (46) has collected similar instances. Herrick (290) found that in some cases pregnancy would continue in the guinea-pig after double ovariectomy. In spite of this large amount of evidence showing that the corpus luteum is not essential during the later stages of gestation, many authors have come to exactly the opposite conclusion and find that the removal of the corpora lutea or ovaries at any stage of pregnancy results in the termination of gestation. Blair Bell and Hick (57), Hammond (264), Weymeersch (635), McIlroy (430), and Dick and Curtis (155) have reported that in the rabbit the removal of the ovaries during pregnancy is inevitably followed by the abortion or reabsorption of the foetuses. Mulon (466) and Daels (151) state that ovariectomy at any stage terminates pregnancy in the guinea-pig. In the cow the removal of the corpora lutea was found by Hess (298), by Wester (634), and by Schmaltz (547) to be incompatible with the continuance of pregnancy. Similar results have been described for the goat by Drummond-Robinson and Asdell (171),



for the opossum by Hartman (272), for the spermophile by Drips (170), and for the mouse and the rabbit by Harris (270) and by Corner (127).

Since the opossum is aplacental, Hartman's (272) results are of particular interest in showing that the effect of the corpus luteum is not solely concerned with facilitating implantation. In addition to the above, cases have been recorded by Hammond (264) and Hartman (277) in which abnormal degeneration of the corpora lutea during pregnancy has led to foetal death.

By means of the unilateral sterilization technique (described on p. 176), it has been possible to get very definite results on mice (499). Unilaterally sterilized mice become pregnant quite readily from the untreated ovary, which undergoes considerable compensatory hypertrophy. In such pregnant mice the removal of the untreated ovary containing the corpora lutea invariably results in the termination of pregnancy. The abortion following the operation takes place 24-48 hours later, so that when the operation is performed at the seventeenth day of pregnancy, *i.e.* two days before parturition would normally take place, the operative abortion and normal birth coincide. Removal of the sterilized ovary containing no corpora lutea has no effect on the gestation. From these experiments it may be concluded that in the mouse the corpora lutea are necessary throughout pregnancy until undergoing regression at about the seventeenth day, forty-eight hours before parturition. There is thus considerable discrepancy between the results of different workers in this field, which may to some extent be due to the variation in the methods used to remove the corpora lutea and also to the variety of animals employed. As regards the latter point, it is improbable that closely related species, such as the rat and mouse, would show any great difference in the necessity for the presence of the corpus luteum, and moreover contradictory results have been obtained by different workers on the same species of animal. The weight of the evidence, however, favours the view that the corpora lutea are essential during the whole of pregnancy.

(f) THE FUNCTIONAL RELATION BETWEEN THE CORPUS LUTEUM AND THE INTERSTITIAL TISSUE

Many workers have supposed that the interstitial tissue and the corpora lutea are to some extent functionally interchangeable, and, if the former is derived exclusively from degenerate membrana granulosa, the hypothesis might be supported on the ground that both are of common origin. It has been shown (see p. 157) that functional luteal tissue can be produced from follicles without ovulation, and even from tissue which has never formed part of an organized follicle; hence the normal origin from an ovulated follicle is not essential. Further, luteal and interstitial cells resemble each other to some extent, and there are thus some histological grounds for supposing that the interstitial tissue might function as a corpus luteum.

It is necessary to consider, therefore, if there is any evidence that the four functions of the corpus luteum can be carried on by the interstitial tissue. As regards the inhibition of œstrus, Haberlandt (256) found that the graft of a third ovary into a normal rabbit caused prolonged inhibition of ovulation. Subsequent examination of the graft showed that no corpora lutea were present, but that the interstitial tissue, as is usual in such grafts, was highly developed. The inhibition of ovulation was ascribed to the abundance of interstitial cells.

Pseudo-pregnant development of the uterus has also been described as being initiated by interstitial tissue. Steinach and Holzknecht (595) have described pregnancy changes in the uteri and mammae of virgin guinea-pigs following destruction of the ovarian follicles by X-rays, and they ascribe the result to the action of the large amount of interstitial tissue resulting from follicular degeneration. This explanation was, however, based on the idea that the interstitial tissue (forming the 'puberty gland') is the chief endocrine tissue of the ovary, that it produces the sole ovarian hormone, and that the changes of pregnancy result from increased production of the one ovarian hormone. Their illustrations show a perfectly developed pseudo-pregnant uterus in an irradiated animal possessing no corpora lutea.

Contrary results are found in the mouse, where X-irradiation

does not interfere with the occurrence of œstrus, and pregnancy changes are not usually produced. In a few exceptional animals (508), however, hypertrophy of the mouse uterus is produced by X-irradiation, and is correlated with the occurrence in the sterilized ovary of what Lipschütz and others would call interstitial tissue, but which was considered (84) to have a greater resemblance to luteal tissue.

As regards the stimulation of the mammary glands by interstitial tissue, it is possible to quote the well-known feminization of the male guinea-pig by an ovarian graft. No corpora lutea or normal follicles are found in the graft, so that the feminization is usually attributed to the large amount of interstitial tissue produced by the graft (see p. 78). Since complete development of the mammary glands is found in such feminized males, the interstitial cells would seem to be capable of performing this function of the corpus luteum. This explanation of hyperfeminization of the male is more probable than the hypothesis that it depends on the production of œstrin by the graft.

According to Biedl (65) the human corpus luteum undergoes degeneration at the end of the first half of pregnancy, and its functions are then taken over by the interstitial tissue which is increased during pregnancy by follicular atresia.

On the other hand, interstitial tissue present before puberty clearly performs none of the functions of the corpus luteum. In many animals the interstitial tissue is said to be most abundant before puberty—*i.e.* when none of the characteristic luteal effects have yet appeared. In the rabbit, the changes in the uterus and mammary glands characteristic of luteal activity do not appear before the first ovulation, although the pre-pubertal ovary is full of interstitial tissue.

The whole problem is complicated by the lack of any agreed definition of interstitial tissue and by the uncertain behaviour of atretic follicles. It seems clear that the granulosa cells of atretic follicles in grafted ovaries produce all gradations of luteal and interstitial cells (Steinach, 591), and until it is ascertained whether an absolute difference exists between luteal and interstitial cells further discussion can be of little value.

## CHAPTER XI

### PARTURITION

NUMEROUS theories of the cause of parturition have been put forward, and while many subsidiary factors (separation of the placenta, etc.) no doubt play a part, it is fairly clear that the physiological changes which initiate the act are due to hormonal stimuli.

#### (a) CORRELATION WITH OVARIAN CYCLE

*Conditions in pseudo-pregnancy.* All the evidence tends to show that parturition takes place when some particular stage of the ovarian cycle is reached. Thus, the immediate forerunner of parturition is the retrogression of the corpora lutea of pregnancy. The view that this retrogression of the corpora lutea is the actual cause of parturition is supported by the fact that at the end of pseudo-pregnancy some animals show certain symptoms normally associated with parturition. Thus, the rabbit plucks its fur and makes a nest, while *Dasyurus* also makes a nest and cleans its marsupial pouch. It may be assumed, therefore, that a certain phase of the ovarian cycle is correlated with the occurrence of parturition. No ovarian extracts, however, have been shown to have any really significant direct action upon uterine contraction, so it is probable that some indirect mechanism is at work.

*The relation between œstrus and parturition.* Since in many animals a period of œstrus follows very soon after parturition, it is necessary to discuss the relation between these two events. In the mouse, rat, rabbit, and guinea-pig, œstrus follows within a few hours of parturition. In other mammals, however, a greater delay is found. Experimentally, it has been shown by injection of œstrin, or by the implantation of anterior pituitary



(see p. 169), that the induction of œstrus during pregnancy leads to abortion or reabsorption. It is not improbable, therefore, that the recurrence of the œstrus-producing stimulus, associated with the atrophy of the corpora lutea at the end of gestation, is connected with the mechanism of parturition.

*Prolongation of gestation by luteal stimulation.* Since the atrophy of the corpora lutea is a necessary prelude to parturition, it would seem that the injection of active luteal extracts when this atrophy is beginning should inhibit the onset of parturition. Data on this point appear to be lacking, but a similar experiment, *i.e.* the prolongation of luteal activity, has recently been carried out by Teel on the rat (611). This author stimulated the luteal tissue during pregnancy by the injection of NaOH extracts of the anterior pituitary body (see p. 153 and p. 186), and apparently, as a consequence of this, the period of gestation was lengthened by two to six days. Teel's conclusions are as follows: (*a*) the increase in the gestational period is due to a delay in implantation of the embryos, (*b*) the foetuses eventually die *in utero* owing to the failure of the parturition mechanism, and are expelled still-born, (*c*) this intra-uterine death is due to severance of the placental attachment. The delay in the implantation of the fertilized ovum is contrary to what would be expected, but even if luteinization of the ovary during pregnancy does not make possible a longer development of the foetuses, it clearly interrupts the normal mechanism of parturition.

#### (b) DIRECT ACTION OF THE OVARY ON SPONTANEOUS UTERINE CONTRACTION

In view of the clear connection between the ovarian cycle and parturition, it might be supposed that some ovarian secretion produced at a certain time in the cycle acted directly on the uterus to cause the activity resulting in parturition. This possibility is substantiated by the fact that the isolated uterus shows in its spontaneous contractions a cyclic variation which is correlated with the ovarian cycle. It has not been possible, however, to show that ovarian extracts act on the uterus to a degree necessary to produce parturition.



*Cyclic variation in the spontaneous activity of the uterus.* It is well known that the uterus of the guinea-pig during œstrus shows remarkable spontaneous contractions, which make the organ at that time useless for the standardization of drugs. The cyclic variation in spontaneous contraction of the uterus has been studied in the rat by Blair (68), Frank and co-workers (222), and Clark, Knaus and Parkes (120); and in the sow by Keye (321), Corner (124), Seckinger (554), and Wislocki and Gutmacher (642). The general conclusion reached is that during diœstrus the uterus shows rapid feeble contractions, while œstrus is characterized by fewer but much more powerful contractions. Seckinger, however, obtained rather contrary results from the Fallopian tube, and Clark, Knaus, and Parkes found the variation in the rate of conduction of a contraction to be the most significant factor. Frank and his co-workers (222) state that after ovariectomy the uterus shows contractions similar to those occurring during diœstrus. Knaus (327-9) has extended this type of work to the rabbit uterus during pregnancy. This author has carried out very careful experiments, in which any possible effect of enlargement of the muscle fibres on the uterine properties was eliminated by the use of a sterilized cornu containing no fetuses. His results show that during the first half of pregnancy the uterus is practically inactive owing to loss of contractility, while during the second half there occurs a continuous rise in spontaneous activity, which reaches a climax at parturition. Knaus ascribes this comparative quiescence of the uterus during most of gestation to the action of the corpus luteum.

*Direct effect of ovarian extracts on uterine contraction.* Acting on the idea that the greater amplitude of the spontaneous contractions of the uterus during œstrus is due to the influence of œstrin, various workers have endeavoured to reproduce the effect artificially. This has been attempted in two ways, (a) injection of the ovariectomized or diœstrous female before preparing the isolated uterus, (b) subjection of the isolated uterus to œstrin. Frank and co-workers (222) and Brouha and Simonnet (101-2) state that the contractions of the uterus of the ovariectomized or diœstrous animal can be altered to those of œstrus by preliminary injection of the animal. Fellner (192),

in one of his early papers, stated that contraction of the isolated uterus was caused by the œstrus-producing extract. Laqueur and his co-workers (356) showed that œstrin stimulated the isolated uterus, and Brouha and Simonnet (103) later stated that the contractions of the uterus after ovariectomy can be caused to change to those typical of œstrus by the addition of liquor folliculi to the bath *in vitro*. They ascribe this result to the presence of an oxytoxic substance in the follicle rather than to the action of œstrin.

Fraenkel (212), however, found no effect on the isolated uterus, and Bourne and Burn (79) found a direct action of œstrin on uterine contraction *in vitro* only when the uterus was particularly sensitive. It may also be pointed out that Frank's view of the continued action of œstrin all through gestation (see p. 122) is difficult to reconcile with his results on uterine contraction. In any case it seems certain that the direct effect of

œstrin on uterine contraction is not sufficient to be of any importance in parturition. The action of active corpus luteum extracts on the uterus does not seem to have been investigated.

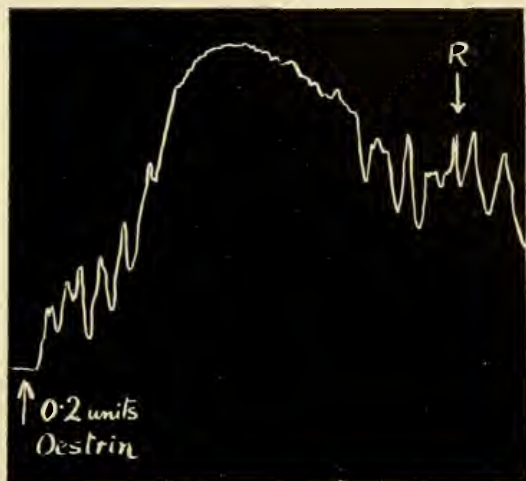


FIG. 68.—EFFECT OF ŒSTRIN ON THE CONTRACTION OF AN ISOLATED GUINEA-PIG UTERUS.

(From Bourne and Burn).

### (c) RÔLE OF OXYTOCIN

Since direct action of the ovary on uterine contraction is insufficient to account for parturition, it is natural to consider if any part may be played by the oxytoxic principle of the posterior pituitary body. Combining the evidence of the correlation of the ovarian cycle with parturition, and the evidence of the action of oxytocin on the uterus, two alternative

theories may be evolved: (a) that the ovary in its immediate pre-partum state exerts a stimulating action on the posterior pituitary which then secretes more oxytocin, or (b) that the immediate pre-partum ovary increases the sensitivity of the uterus to the posterior pituitary.

*Influence of the ovary on the posterior pituitary body.* With the former of these ideas in view Dixon and Marshall (161) investigated the effects on the posterior pituitary body secretion of extracts of ovaries at various stages of the cycle. These experiments were performed upon the dog, and consisted in the collection of the cerebro-spinal fluid before and after the injection of ovarian extracts, and in the assay of the cerebro-spinal fluid samples for their oxytoxic action. As a result of these experiments, Dixon and Marshall concluded that the ovary at a certain stage of its cycle, when the corpora lutea were in regression, elaborated some secretion which stimulated the posterior pituitary body. They considered a similar action at the end of pregnancy to be a vital factor in the mechanism of parturition. Many criticisms have, however, been levelled against these experiments, for instance by Swale Vincent (623). In the first place, doubt exists as to whether the posterior pituitary body actually secretes into the cerebro-spinal fluid, and in any case Dyke and Kraft (174) found no changes in the oxytoxic properties of the cerebro-spinal fluid during labour. Secondly, various tissue extracts may affect the secretion of the posterior pituitary, and thirdly, Blau and Hancher (69) entirely failed to confirm Dixon and Marshall's results. Addis (1), however, appears to have obtained some clinical results in keeping with the hypothesis.

*Effect of the corpus luteum on sensitivity of the uterus to oxytocin.* The evidence that the pre-partum ovary stimulates the secretion of the posterior pituitary is not, therefore, conclusive. On the other hand, the evidence that the uterus has a cyclic susceptibility to oxytocin does seem to be conclusive. Knaus (326) found that abortion can only be produced in the rabbit by the injection of posterior pituitary substance during the last few days of pregnancy; earlier than this no result follows the administration of even large amounts. In confirmation of this result, Knaus (328), as an extension of the work referred to



earlier (see p. 202), has shown that the uterus of the rabbit is practically insensitive to oxytocin during the first eighteen days of pregnancy. During the second half of pregnancy the sensitivity increases slowly, until just before parturition, when the increase is very rapid.

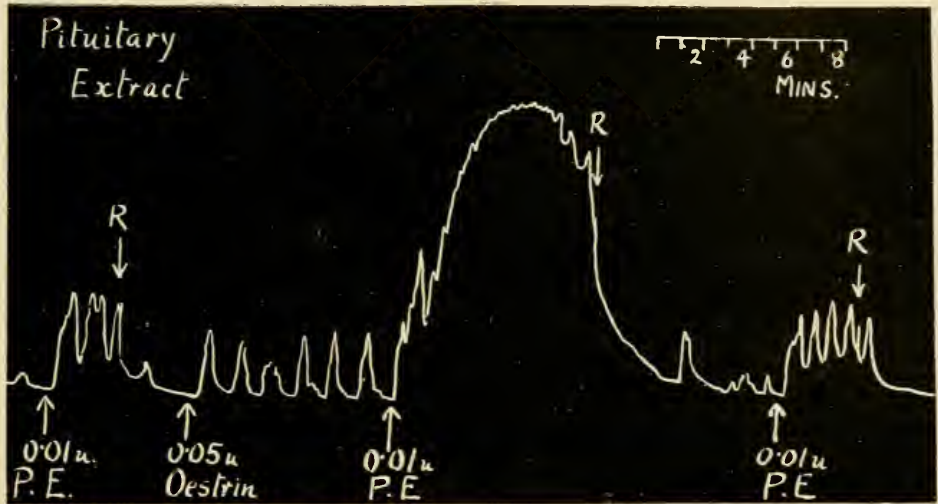


FIG. 69.—ACTION OF ŒSTRIN IN SENSITIZING AN ISOLATED UTERUS TO OXYTOCIN.

(From Bourne and Burn).

On Knaus' view, therefore, parturition is due, at least in part, to the uterus developing a far greater sensitivity to oxytocin towards the end than is found during the rest of pregnancy. This insensitivity during the major part of pregnancy Knaus ascribes to the action of the persistent corpora lutea of gestation.

*Effect of œstrin on sensitivity of the uterus to oxytocin.* In comparison with Knaus's work on the effect of the corpus luteum, interesting experiments have recently been performed on the effect of œstrin on uterine sensitivity. Brouha and Simonnet (102) and Miura (461) have shown that the preliminary injection of the animal with œstrin greatly increases the sensitivity of the uterus to oxytocin. Bourne and Burn (79) extended this result by showing that the initial treatment of the uterus *in vitro* with œstrin greatly increases the response to oxytocin. Actually a synergism exists between œstrin and oxytocin.

It is clear, therefore, that the decline of the corpus luteum and the reassertion of the œstrus-producing stimulus both tend to increase the sensitivity of the uterus to oxytocin. It is difficult to estimate at the moment just how important this effect may be in the causation of parturition, but it would seem that it must be a highly important, if not crucial, factor.

(d) RELATION OF PARTURITION TO EFFECTS OF  
OVARIECTOMY DURING PREGNANCY

It is interesting to consider the effects of double ovariectomy and removal of corpora lutea during pregnancy in connection with the theories of the mechanism of parturition. The fact that double ovariectomy leads to abortion means that either this effect is not comparable to true parturition, or else that parturition is not a positive ovarian action. In other words, if the experimental abortion is comparable to parturition it is difficult to explain how the latter, as on Dixon and Marshall's results, can be due to some internal secretion of the ovary. If, on the other hand, Knaus's work is accepted as the basis of a theory of the mechanism of parturition, it is clearly possible to compare the abortion which follows ovariectomy or removal of corpora lutea during pregnancy with true parturition. Assuming that the corpora lutea have a de-sensitizing effect on the uterus, their removal by ovariectomy would permit the reappearance of uterine sensitivity to oxytocin; if the corpora lutea only were removed the sensitivity would be increased by the action of the œstrus-producing hormone of the ovarian stroma. On the whole, therefore, it seems probable that the termination of pregnancy which follows removal of the corpora lutea or double ovariectomy can be compared to some extent with true parturition. This, of course, only applies to the later stages of pregnancy. The interruption of pregnancy in the early stages, either by ovariectomy, removal of corpora lutea, or injection of œstrin, is probably due to adverse uterine changes.

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